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THE QUEENSLAND JOURNAL OF AGRICULTURAL SCIENCE

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JUNE, 1952.

# CHRONIC ENDEMIC FLUOROSIS OF MERINO SHEEP IN QUEENSLAND.

By J. M. HARVEY, M.Sc., Senior Chemist, Biochemical Section, Chemical Laboratory, Division of Plant Industry.

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## SUMMARY.

*1. The distribution in Queensland of underground waters containing various amounts of fluorine is mapped.*

*2. Levels of fluorine toxic to Merino sheep are largely confined to thermal bicarbonate waters.*

*3. Transmission studies indicate that there is little or no transmission of fluorine from the ewe to the foetus on waters containing up to 10 p.p.m. fluorine and that no appreciable amount of fluorine is transmitted through the milk.*

*4. Dietary mitigation studies show that the feeding of diets high in protein, calcium or phosphate is not effective in combating fluorosis in sheep on waters containing 5 and 10 p.p.m. fluorine.*

*5. Fluorine in the drinking water affects fleece weight and quality only indirectly, through its impairment of grazing efficiency of the animal.*

*6. Storage of fluorine in the edible portions of the sheep after two years on water containing up to 10 p.p.m. fluorine is not high enough to constitute a danger to humans.*

*7. Teeth and bone lesions associated with fluorosis are illustrated. Rarification rather than thickening of the bones is shown.*

*8. There appears to be no economical method of reducing the fluorine content of stock waters in Queensland.*

*9. Methods of management of various classes of sheep which will minimise the harmful effects of fluorine are suggested.*

## INTRODUCTION.

Chronic endemic fluorosis is a disorder associated with the intake of toxic levels of fluorine.* It affects all classes of domesticated animals as well as man. In Queensland, the high fluorine level of many waters, chiefly from artesian and sub-artesian bores, in the Great Artesian Basin (see Appendix) is responsible. The industry most affected is Merino sheep production.

The most obvious manifestations are the dental abnormalities which occur in animals continuously exposed to fluorided water. The teeth are modified in colour, size, orientation, shape and structure. Reduction in hardness results in pitting of the incisors, often in a definite horizontal pattern, and selective abrasion of the molars. The teeth wear unevenly through chipping and/or snapping at the lines of pitting. The mandible may become thickened, either by isolated exostoses or by a diffuse covering of periosteal bone, and in severe cases other bones may be similarly affected. With continued high fluorine intake, weakness and impairment of the use of the limbs and thickening of the joints are apparent. Ockerse (1946) and Lyth (1946) described identical symptoms in man similarly exposed. The clinical picture was stiffness of the spine with pain in the lumbar region, and pain and stiffness of joints of the upper and lower limbs. Ossification of the vertebrae and ligaments, with osteophitic growths and periosteal deposits on other bones and joints, has been recorded.

In livestock in Queensland the most serious effect of fluorosis is the damage to incisor and molar teeth, for in advanced cases the animals are unable to gather or masticate the harsh fodder of pastoral areas during the inter-monsoonal period and they may become less productive or even succumb. The pain throughout life, in the case of badly affected animals, could be a major factor in reducing wool and meat production. The shortened breeding

* The specific term "fluorine" will be used in this paper. It will be understood that the free element, fluorine, does not occur naturally and that fluorosis is associated with the intake of fluorine in a combined form such as sodium fluoride.

life of ewes and the increased mortality of lambs at birth or soon after contribute to a slow natural increase in flock numbers. During drought, when food becomes scanty and more fibrous plants are eaten, the effects of fluorosis may be greatly accentuated.

The disease has been recognized in Queensland only since about 1940, and its importance is perhaps not yet fully realised. It had been known for some time prior to the first recording of dental defects due to fluorosis in 1941 that wool production was lower in areas now known to be endemic, but a disorder such as fluorosis had not been suspected. One reason for this may have been that it was not common to examine the mouths of sheep, as there was little need to drench for worm control and sales of sheep were not common because of low lambings and low wool production. Further, many large properties had not then been subdivided and sheep would have had access to more than one source of water, with a consequent reduction of the severity of dental lesions.

The significance of the disorder to the sheep industry in Queensland was stressed by the Agricultural Chemist of the Department of Agriculture and Stock in the Annual Report of the Department for the year 1945-46 (White, 1946). He stated, as a result of preliminary surveys: ". . . . the seriousness of the position had not been underestimated. It is felt that the more or less stationary level in Queensland sheep numbers is in no small measure due to the very wide distribution of fluorided underground waters."

## HISTORY.

Fluorine, the most chemically active of the halogens, was isolated by Moissan in 1886. It is estimated to constitute approximately 0·1 per cent. of the first half-mile of the earth's crust. Its most abundant occurrence is as a constituent of fluorspar ($CaF_2$) and cryolite ($3NaF,AlF_3$), but it is also present in such minerals as biotite, tourmaline, sellaite ($MgF_2$), phlogopite, muscavite and fluoapatite. Rock phosphate, the raw material used in the manufacture of commercial superphosphate, may contain over 4 per cent. of fluorine.

The earliest records of fluorine poisoning in domestic animals are associated with volcanic eruptions. In Iceland, in 1100, losses were recorded in sheep. Losses also occurred in the eruption of Hekla in 1845, and in the following year emaciation, decreased milk yield, weakness and impairment of the use of limbs, thickening of the joints and development of exostoses of long bones and jaws became apparent in surviving animals. Mottling of the teeth, with selective abrasion of the molars and pitting of the incisors, was also described. These findings were recorded by Roholm (1937a), who, by examination of museum specimens preserved since 1845, was able to show that the disease was a chronic fluorine intoxication.

The first mention of "mottled enamel" was probably made by Kuhns (1888), who observed black spots on the teeth of people in Mexico. Similar conditions were recorded in Italian emigrants (Eager, 1901).

The occurrence of a disease similar to that recorded in Iceland has been reported among animals grazing in the neighbourhood of various factories. The industries principally concerned are those producing superphosphates, hydrofluoric acid, glazed bricks, copper, aluminium, glass and enamel. Bartolucci (1912) first ascribed this disorder to fluorine. He stated that an occurrence of the disease in cattle was associated etiologically with fluorine-containing gases emanating from the factory, and that water from a well sunk near the canal carrying the factory effluents contained fluorine. A description of the disabilities suffered by animals in the neighbourhood of a factory at Freiburg, Saxony, which utilised fluospar as a flux, was given by Haubner (1878). The earliest fluospar fluxes were made from the best calcium fluoride—this would mean the maximum liberation of hydrofluoric acid and would explain the high incidence of fluorosis noted in the early records.

The disease was first described in the United States of America as occurring in humans at Colorado Springs (Fynn, 1910). McKay and Black (1916) postulated that the etiological factor was some rare element present in the drinking water during the period of calcification of the teeth. This conclusion was reached after studies had shown that the incidence of mottled teeth was limited to certain well-defined geographical areas and there only to those individuals exposed during the years of enamel formation. It was also found that after the water supply of Colorado Springs was changed no more cases of mottling occurred. It was not until 1931 that Smith, Lantz and Smith (1931), Churchill (1931) and Velu (1931), working independently, proved beyond doubt that fluorine in drinking water causes mottled enamel.

The disease has now been reported from practically every country in the world. The trouble known as "darmous," which occurs in horses, cattle and sheep as well as in humans in Algeria, Morocco and Tunis, has been shown to be due to poisoning by fluorine (Velu, 1932; Gaud, Charnot and Langlais, 1934). Dean and McKay (1939) reported 375 known endemic areas in U.S.A. Cases have been reported from Canada by Walker and Spencer (1937); from Mexico by Kuhns (1888) and Mazzotti and Gonzalez (1939); from the Argentina by Chaneles (1932), Munoz (1934) and Erausqin (1934, 1935); from adjoining countries in South America by Damon (1930); from North Africa by Velu (1932, 1933, 1934, 1938); from South Africa by Brown (1935), Raubenheimer (1938), Staz (1938), Steyn (1938-39) and Ockerse (1941, 1946); from Japan by Masaki and Mimura (1931); from China by Anderson and Stevenson (1930), Anderson (1932), Ni (1937) and Lyth (1946); from Java by Liang (1939); from India by Shortt *et al.* (1937), Shortt, Pandit and Raghavachari (1937), Pillai (1938), Wilson (1939), Day (1940), Raghavachari and Venkataramanan (1940) and Pandit *et al.* (1940); from England by Ainsworth (1933), Morgan (1939) and Wilson (1939); from Scotland by the Medical Research Council (1949); from Italy by Ricci (1933); from Hungary by Straub (1904); from Bahama Islands, the Barbadoes, Cape Verde Islands, Spain and Holland by McKay (1930); and from Greece and neighbouring islands by Lambadarides (1940, 1941) and Koutsouveli (1940).

Its occurrence in Australia was first officially noted in 1937 by Clements (1939), who observed the typical lesions in humans from two Queensland towns. White (1944), Seddon (1945) and Moule (1945) found the dental lesions in all stages among sheep using certain bore waters as their only drinking supply.

## RECORDED EXPERIMENTAL OBSERVATIONS.

The effects of continual ingestion of fluorine from a variety of sources have been investigated in cattle, sheep, goats, pigs and poultry. Peirce (1939) reviewed the experimental findings up to 1939. In all experiments animals showed reduced appetite and less efficient utilisation of food when the ingestion of sublethal amounts of fluorine was continued. This resulted in less rapid growth in the young and loss of weight in mature animals. Unthriftiness, anorexia and death followed the ingestion of relatively large amounts of fluorine. Specific chemical and morphological changes were manifested in the bones and teeth. The normal ivory colour of the bones changed to chalky white and the diameter of the cross-section of the long bones was increased, due in part to an enlargement of the marrow cavity but mainly to an increase in the thickness of the bone substance. Exostoses of the long bones and of the jaws were a common feature. The teeth, particularly the incisors, became pitted and eroded. The molars became abraded and uneven wear seriously hindered mastication. In some cases the pulp cavities were exposed, either by fracture or by wear, and this led to considerable pain with all its consequences. Few, if any, dental changes occurred if the teeth had been fully developed prior to the ingestion of fluorine, although bone changes could be induced at any age.

It has been shown that normal bone tissue contains some fluorine, and it may well be that this element is a necessary constituent of healthy bone. However, the continued ingestion of fluorine at relatively high levels increases enormously the fluorine content of bone and tissue as well as of some other organs and body fluids (e.g., thyroid and blood). Atrophy of the spongiosa, defective and irregular calcification of the newly formed osseous tissue and active periosteal bone formation, which is considered to resemble the histological picture of osteomalacia, have been described in the bones of pigs, cattle and sheep suffering from the chronic effects of ingestion of fluorine.

Comparison may be made between the deposition of phosphate and that of fluorine. Both are preferentially deposited in the long bones. Neuman *et al.* (1950) showed that, at low concentration, fluorine replaces the hydroxyl and bicarbonate group and is stored in the bone as calcium fluoride. In addition, fluorine acts as an enzyme poison at the point of access. The mechanism that the body adopts to remove excess fluorine is similar to that for

the detoxication of lead, both being taken out of circulation by deposition in bone. Removal of lead from bone is now possible by de-leading treatment, but fluorine does not appear to be released by any method.

Other effects of fluorine poisoning which have been described are reduced milk yield in cattle and reduced egg production in poultry, both effects being no doubt correlated with lowered food intake. Both diuresis and increased water consumption have been observed, with diarrhoea as a frequent symptom. Anaemia and photophobia have been reported. Calcium and phosphorus retention and levels of serum calcium and phosphorus have been found to vary with different species of animals. Early eruption of the permanent incisors has been observed in sheep.

The detrimental effects of fluorine in animal nutrition have been established by a number of investigators. Reviews have been published by McClure (1937) and Mitchell (1942).

The possible toxic levels of fluorine in mineral mixtures for livestock has received considerable attention. The Association of American Feed Control Officials, in its official publication (1942), proposed that mineral mixtures for sheep should contain not more than 0·35% fluorine and that the concentration in the grain ration should not exceed 0·01%. Peirce (1938), using breeding ewes without access to pasture, concluded that the toxic level was between 0·011% and 0·019% of the total dry ration. Mitchell (1942) concluded that the fluorine concentration in the total dry ration of sheep should not exceed 0·003%, but he made no reference to the special requirements of lambs and breeding ewes.

Shrewsbury *et al.* (1944) investigated the tolerance of growing lambs for fluorine contained in rock phosphate added to a grain supplement. The basic ration was lucerne and clover hay in winter and blue grass pasture in summer. The results may be summarized as follows:—

(1) For lambs, the maximum safe level of fluorine in rock phosphate was 1·5 to 3·0 mgm. per kilogram body weight daily. The standards used were teeth and bone characteristics.

(2) For breeding ewes, all levels of fluorine had some effect on maintenance in the second and third years after exposure to fluorine.

(3) The growth of lambs was not affected by the fluorine ingested by ewes, nor was birth rate or birth weight.

(4) Wool production was not affected.

(5) Fluorine plays some part in iodine assimilation, increasing the storage of iodine in the thyroid. Iodine does not counter the deleterious effects of fluorine.

A number of factors appear to have been overlooked by most workers in their studies on the harmful levels of fluorine in mineral mixtures. These include:—

(i) The percentage of fluorine in the ration means little unless correlated with both total intake and the form in which fluorine is fed—this was considered by Shrewsbury *et al.* (1944).

(ii) The findings may not necessarily be applicable to all breeds of sheep.

(iii) The influence of the type of ration or pasture could be important.

(iv) There is a possibility of transmission of fluorine through foetal circulation or through milk.

These factors could well explain the wide variation in recorded toxic levels of fluorine in mineral mixtures.

The toxicity of fluorine as a fluoride in solution appears to be greater than when the same salt is consumed in the solid state. Velu (1932) recorded that a daily intake of 0·5 mgm. of fluorine per kilogram body-weight in water was as toxic to sheep as rock phosphate mixed with the ration in sufficient quantities to supply 20 mgm. daily. Marcovitch and Stanley (1938) established a marked difference in the cumulative effect of fluorine in rats consuming fluorine in the drinking water and those with the same fluorine intake in the ration.

No attempt has been made to define the limits of fluorine in water which will produce symptoms in sheep. In fact, minimal levels of fluorine in the drinking water which will be harmful are not known for any animal, domestic or otherwise.

It is apparent from clinical observations in affected areas that man is more susceptible than sheep. Van der Merwe (1940) stated that quantitative epidemiological-chemical studies by the American Public Health Service showed that mottling of the permanent teeth in man is caused by using water containing fluorine in excess of 1 p.p.m. for both cooking and drinking purposes during the period of dental susceptibility. Sugawa (1938) observed a high incidence of mottled teeth amongst the inhabitants of the island of Sakishima using water containing 0·7 to 1·0 p.p.m.F, and in people in the Kagoshima perfecture of Japan on 0·3 p.p.m.F. Zelmanova, Forst and Shafir (1937) reported that, on water containing 0·02 to 0·9 p.p.m. F, 6% of children showed calcareous spots on the teeth.

Van der Merwe (1940) observed no visible effects on the calcification of teeth of cows, goats, sheep and donkeys of water containing up to 8 p.p.m.F. Velu (1938) stated that water containing 7 p.p.m.F as calcium fluoride did not affect the teeth of animals. Steyn (1937) stated his belief that a fluorine content of at least 4 to 6 p.p.m. in the drinking water is necessary to cause enamel defects in animals. His observations were made on stock during the

susceptible period when permanent teeth were being laid down, but apparently no consideration was given to the influence of season or diet on the incidence of the disease.

There are indications that mild symptoms in Queensland can be noticed in sheep on much lower levels of fluorine than those quoted above. Moule (1945) stated that water containing 2 p.p.m.F produces lesions in the teeth of lambs. Goats and sheep running on a town common in western Queensland, and watered by a bore containing 2·4 p.p.m.F, were found to show mild fluorosis. Sheep on a water which contained 0·5 p.p.m.F at the borehead (see Appendix) showed large white teeth and mild fluorosis.

The difference in the opinions expressed on the minimal fluorine levels in drinking water necessary to produce symptoms of fluorosis in sheep under field conditions is due to a number of factors. These may be summarized as follows:—

(1) The age of sheep when they first have access to fluorided water. Lambs are most vulnerable; grown sheep are almost unaffected by fluorine levels in the drinking water which would produce serious effects in lambs.

(2) The duration of access to and protection from fluorided water. On many properties in endemic areas, surface water is available for some period in most years.

(3) The rate of water evaporation. The concentration of fluorine at a borehead is generally much less than at distances along the bore drains, in dams or in drinking troughs.

(4) The prevalence of succulent feed. This depends on the season and greatly influences the animals' water consumption.

(5) Other possible sources of fluorine, such as plants containing fluorine.

(6) The quantity and composition of the feed.

## THE OCCURRENCE OF FLUORINE IN WATER IN QUEENSLAND.

Figs. 1–6 show the distribution in Queensland of underground water containing fluorine. The maps have been drawn up in terms of bore blocks, each of which is the area enclosed by one degree of latitude and one degree of longitude. Each marking within a bore block indicates a water sample from that bore block but not the site from which the sample was drawn.

The average as shown in these maps is somewhat fortuitous in that more samples have been submitted from some blocks than from others. From the unmarked blocks, no samples have been taken. Underground waters outside the Great Artesian Basin have also been included, as isolated cases of such waters containing fluorine have been found.

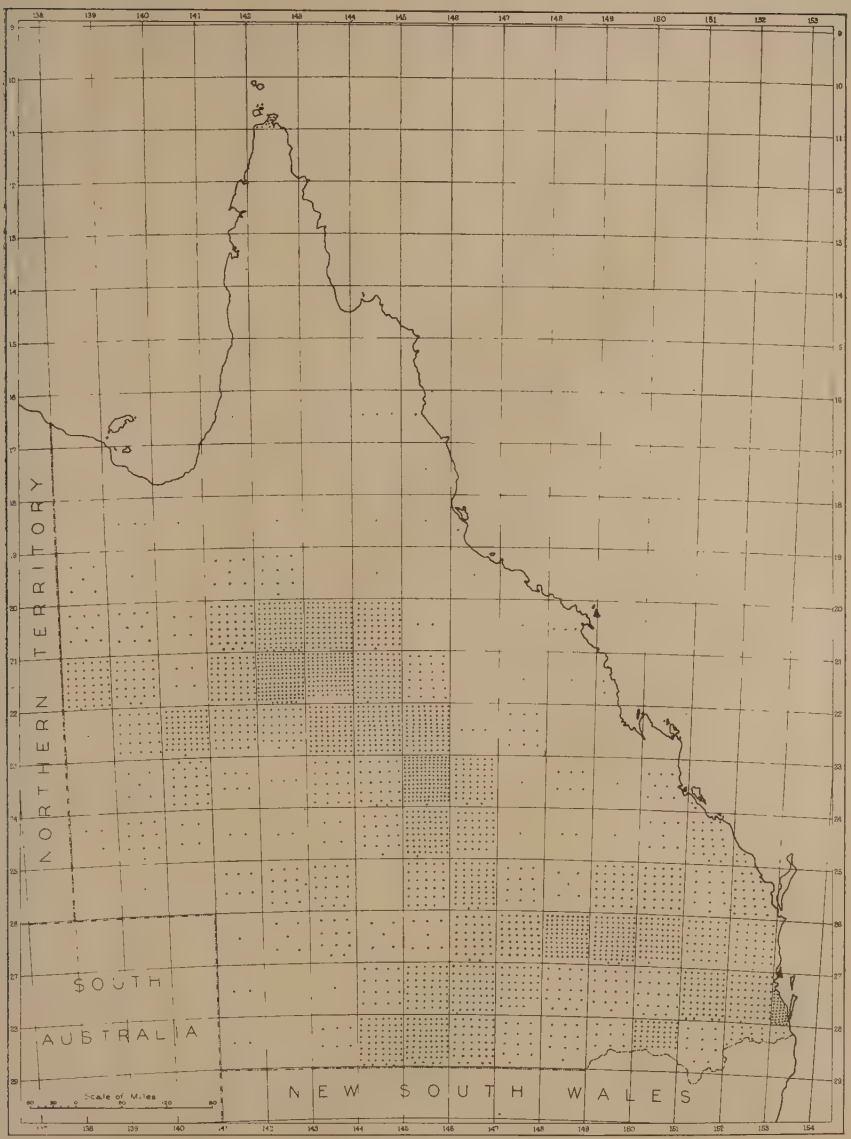



**Fig. 1.**

Distribution of Waters Analysed and Found to Contain Less than 1 p.p.m. Fluorine.

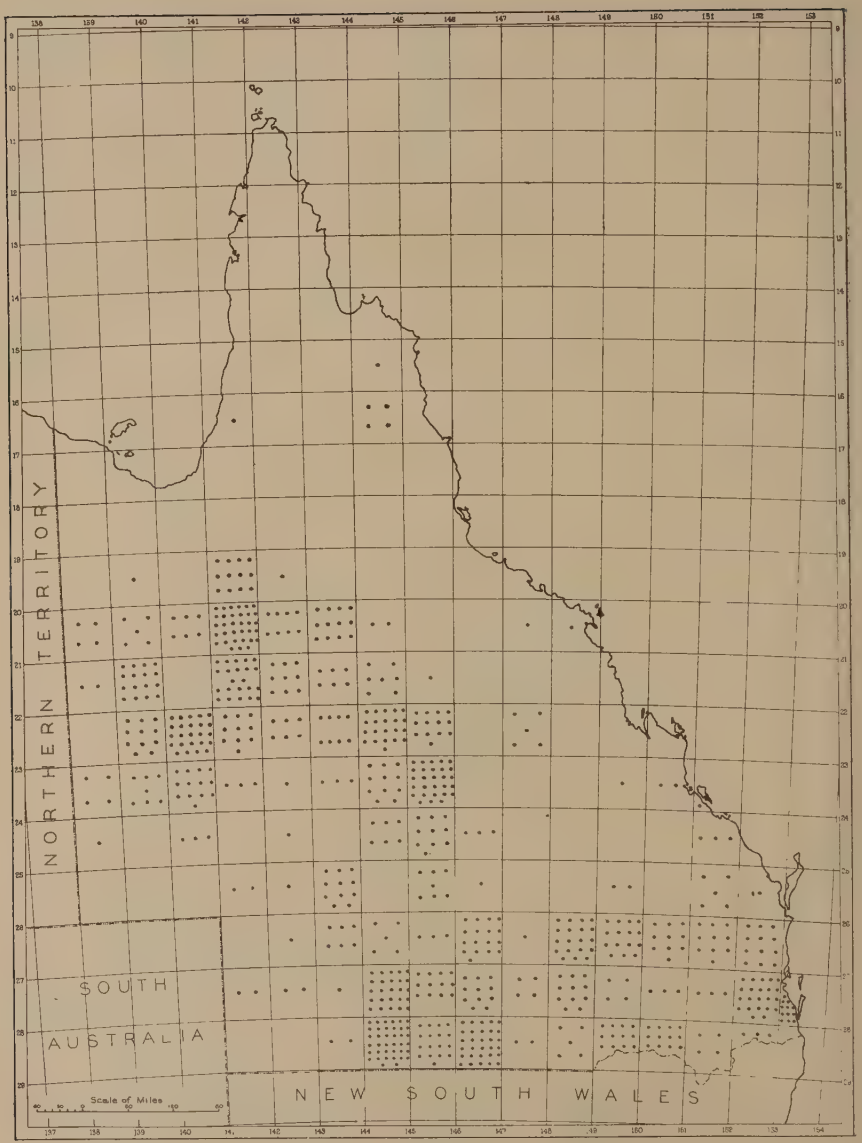



Fig. 2.
Distribution of Waters Analysed and Found to Contain 1–2 p.p.m. Fluorine.

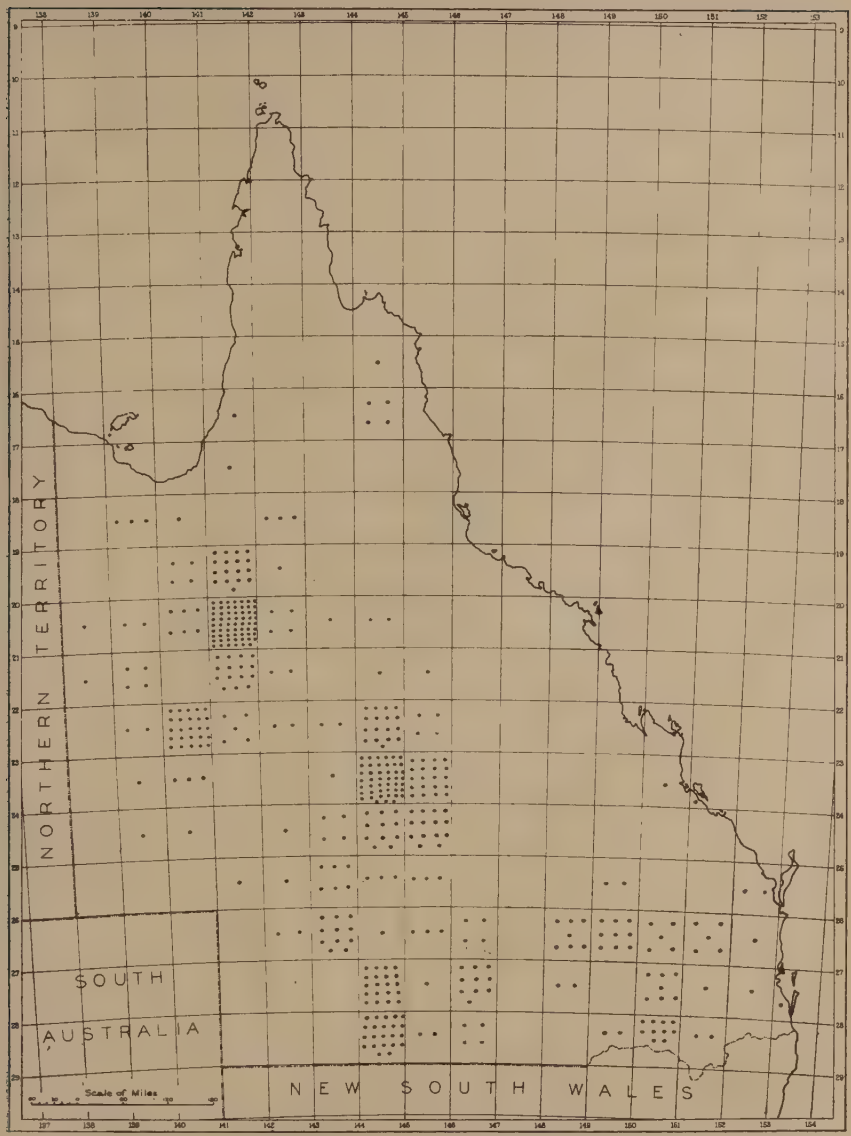



Fig. 3.
Distribution of Waters Analysed and Found to Contain 2–5 p.p.m. Fluorine.

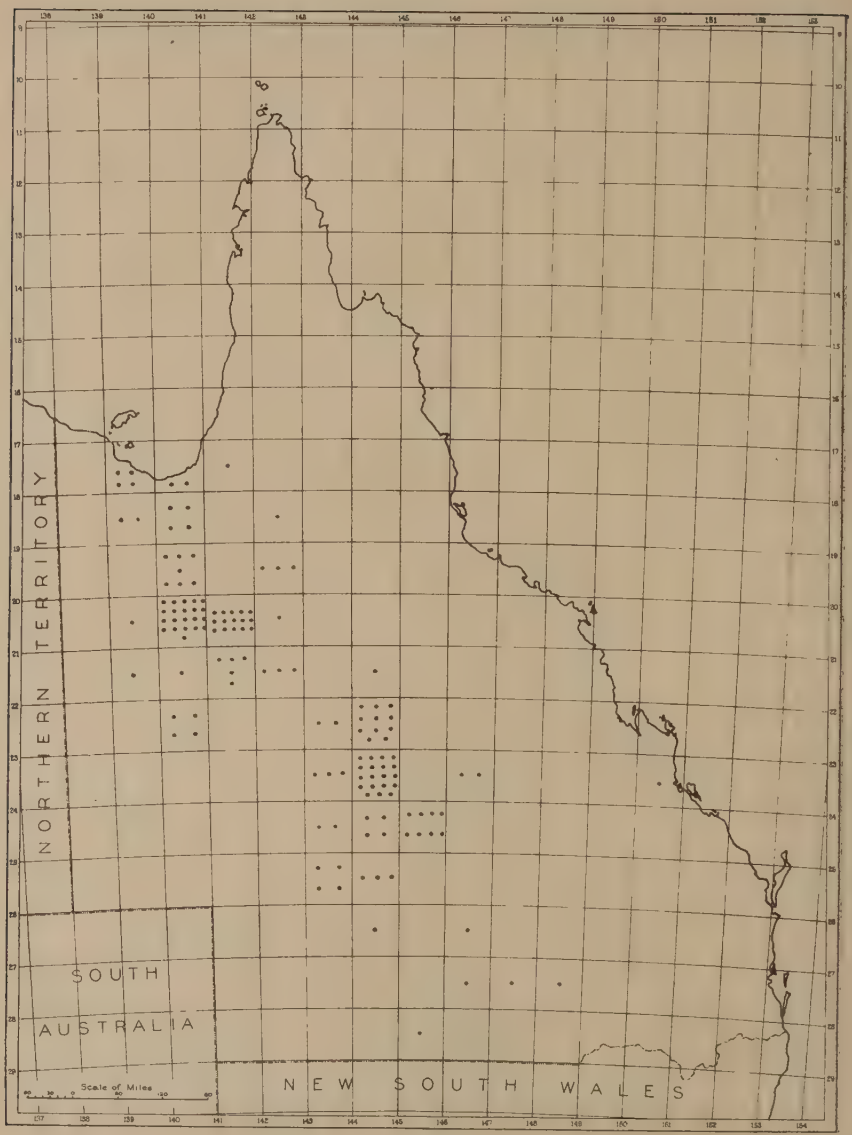



Fig. 4.
Distribution of Waters Analysed and Found to Contain 5–10 p.p.m. Fluorine.

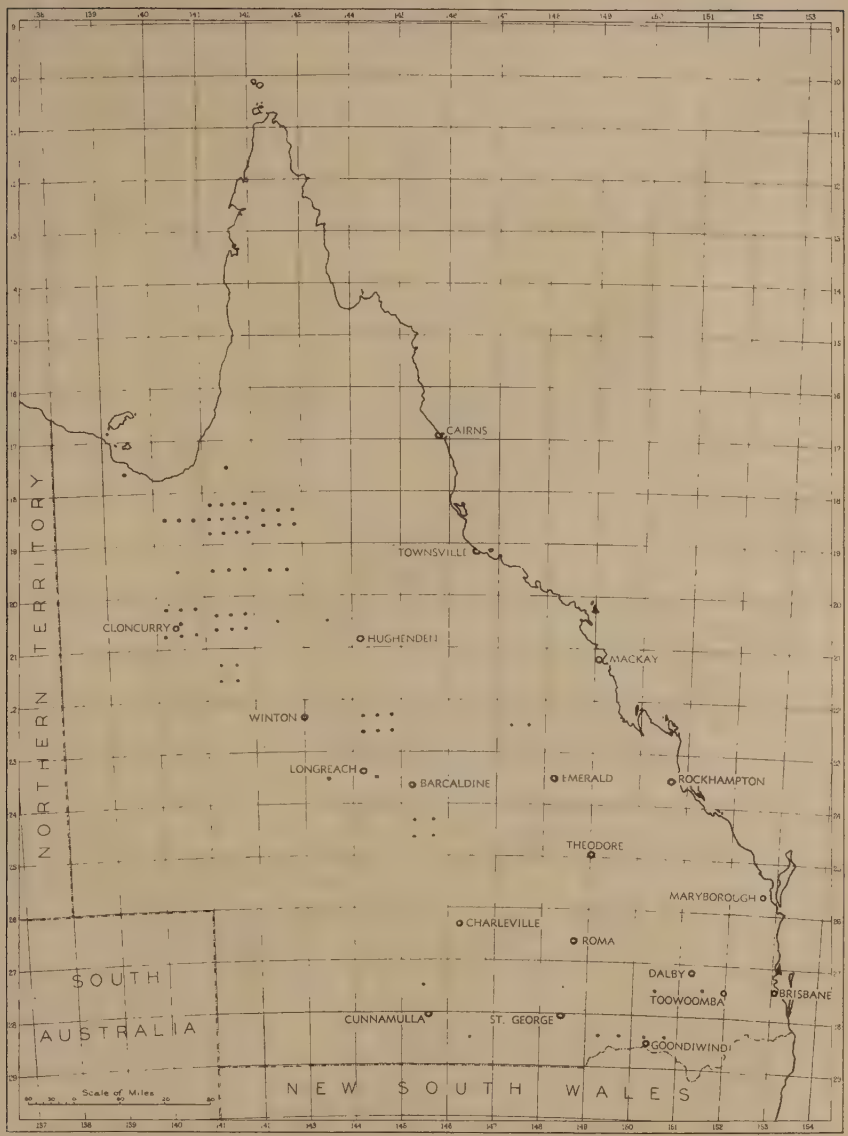



Fig. 5.

Distribution of Waters Analysed and Found to Contain More than 10 p.p.m. Fluorine.

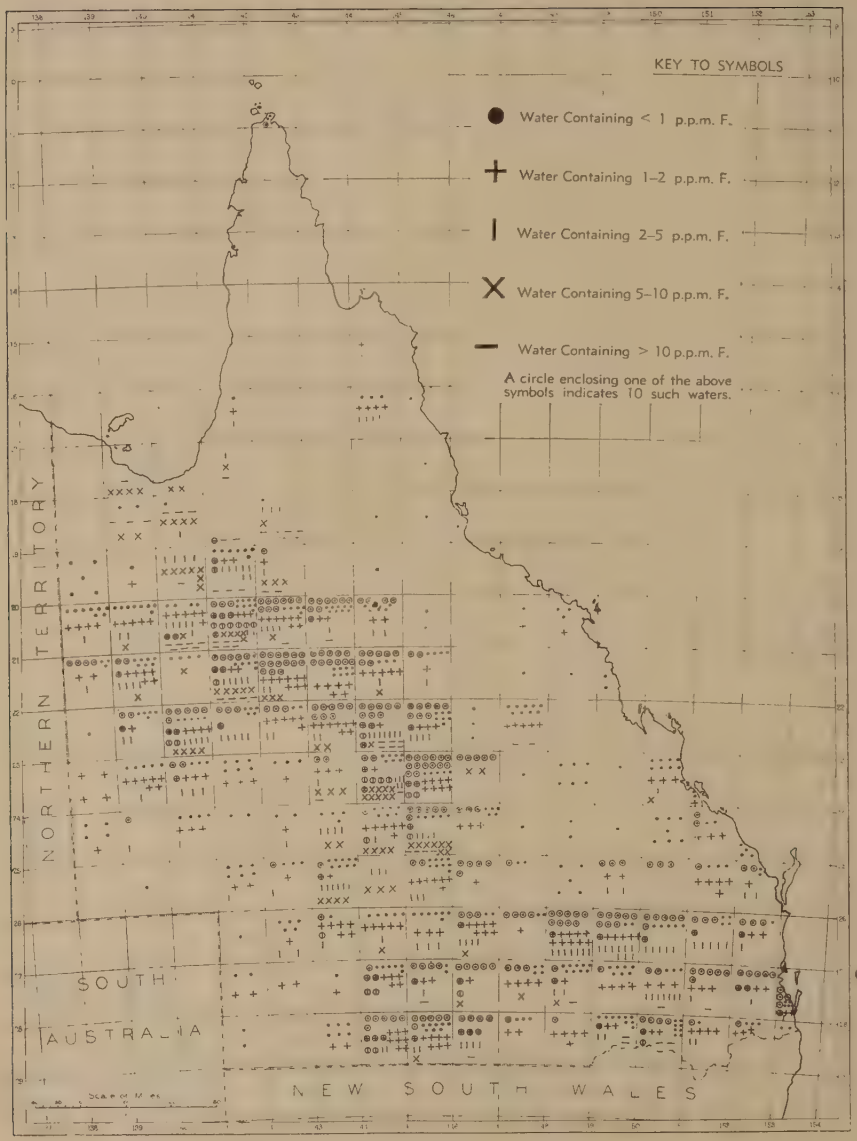



Fig. 6.

Proportions of Waters of Various Fluorine Contents in Each Bore Block.

Bond (1946) made the following statement regarding fluorided waters in South Africa:—"It is only the Red Granite water and water issuing from the Pilansberg alkali rocks that consistently contain fluorides over 1 p.p.m. Some Old Granite waters do contain a high fluoride level but this is not a consistent feature. There are a few isolated cases where high fluoride has been found in waters arising in other formation. Also with very few exceptions only waters which contain sodium carbonate or sodium bicarbonate have an appreciable quantity of fluoride. The converse is not necessarily true. Fluorite and fluo-apatite is a common accessory mineral in the Red Granite and Pilansberg alkali rocks."

McKay and Black (1916) noted that at Colorado Springs, in the U.S.A., where mottled enamel is recorded, the geological formation is also granite.

Lindgren (1933) stated that fluorine is present in many waters, both superficial and deep, but it appears in larger quantities in waters of the sodium carbonate type.

Shortt, Pandit and Raghavachari (1937) showed that in India zones of endemic dental fluorosis are underlain by granite or gneiss.

Whitehouse (1947) discussed the following three known extensive areas of high fluorine concentration in Queensland:—

(*a*) About Eulo-Hungerford; lat. 28°–29° long. 144°–145°.

(*b*) About Cloncurry-Julia Creek; lat. 20°–21° long. 140°–142°.

(*c*) North and east of Blackall; lat. 24° long. 145°–146°.

Areas (*a*) and (*b*) were reported to be close to and probably genetically related to bedrock granites. Of area (*c*), where fluorides occur in all sedimentary series, it was suggested that there is a persistent source (not yet obvious) somewhere to the east which has shed fluoride minerals during a long period of time. Additional areas of high fluoride content are at Roma (granite basement), Longreach (granite basement), and the extensive shed from the igneous complex in the area between Hughenden and Croydon. The following conclusions were drawn from the examination of the geology of area (*b*) in the light of analytical data supplied by the Department of Agriculture and Stock:—

(1) Fluorides are associated with granite bedrock or with primary granitic detritus little dispersed from its source.

(2) Fluorides are produced *in situ* in the presence of sodium carbonate or bicarbonate but do not seem to be related to the absolute quantity of carbonate.

(3) Temperatures are of the order of 100°F.–150°F.

(4) Fluorides occur when a slowing down of pressure water percolation could be expected—at the bottom of a structural slope on the one hand and in the poorly flushed elevated confined ends of aquifers on the other.

(5) The fluorine content of the bores is extremely uneven, varying from nil to 14 p.p.m., indicating that they are produced *in situ* and not transported for any appreciable distance without marked dilution.

(6) The provenance of this sediment could supply the necessary fluorine source minerals — fluorspar, apatite and possibly cryolite.

(7) Fluorides are then of limited occurrence and distribution, produced where a combination of favourable factors co-exist—detrital granite source, sufficient sodium carbonate or bicarbonate concentration, appropriate temperature and pressure and restriction of ground-water flow to allow sustained contact of source and attacking solutions and restricted dispersal of fluorides when produced.

While it is logical to conclude that the fluorine present in artesian water comes from parent source minerals containing fluorine as calcium fluoride, this does not explain the occurrence of underground water containing fluorine in excess of 8 p.p.m. From solubility considerations, it is inconceivable that a level in excess of 8 p.p.m.F. in solution could occur as calcium fluoride. The fact that fluorine is associated very largely with thermal bicarbonate waters tends to indicate that bicarbonate at high temperatures and high pressures, for long periods of time, plays some part in the conversion of parent calcium fluoride to a more soluble fluoride salt, a procedure which cannot be explained by simple chemical reactions under laboratory conditions. Some system of ionic exchange involving sodium hydroxide progressively formed from sodium bicarbonate at high temperature would appear to be highly probable. Analogy could be drawn with the production of sodium fluoride in solution in the regeneration of spent bone by means of sodium hydroxide solution. Here a specially prepared product is used, on a small scale, for the removal of fluorine from affected water and regeneration of the product is effected by means of sodium hydroxide.

Examination of analytical data for the waters analysed in connection with the Fluorine in Water Survey conducted by an inter-Departmental Committee appointed by the Queensland Government reveals that toxic fluorine levels are largely confined to thermal bicarbonate waters, but such waters are not necessarily heavily fluorided. Unlike those of South Australia (Ward, 1945), a number of waters in Queensland have been found to contain both sulphate and fluorine.

## INVESTIGATION OF FLUOROSIS IN QUEENSLAND.

In 1946 it was decided that research into some features of fluorosis in Merino sheep should be made. Although a considerable number of water analyses had been carried out, some endemic areas mapped, and most of Whitehouse's (1947) hypotheses postulated, no information was available on the following:—

(1) The transmission of fluorine by ewes drinking toxic fluorided water either (*a*) through foetal circulation, or (*b*) to the growing lamb through the milk.

(2) The possible alternative sources of fluorine to sheep in endemic areas.

(3) The effect of various rations on the onset of symptoms of fluorosis in sheep drinking fluorided water.

(4) The accumulation and the excretion of fluorine by sheep using fluorided water.

(5) The accumulation of fluorine in keratinous tissue as a possible index of the degree of exposure of sheep to water containing fluorine.

(6) The practical elimination of fluorine from affected water in endemic areas.

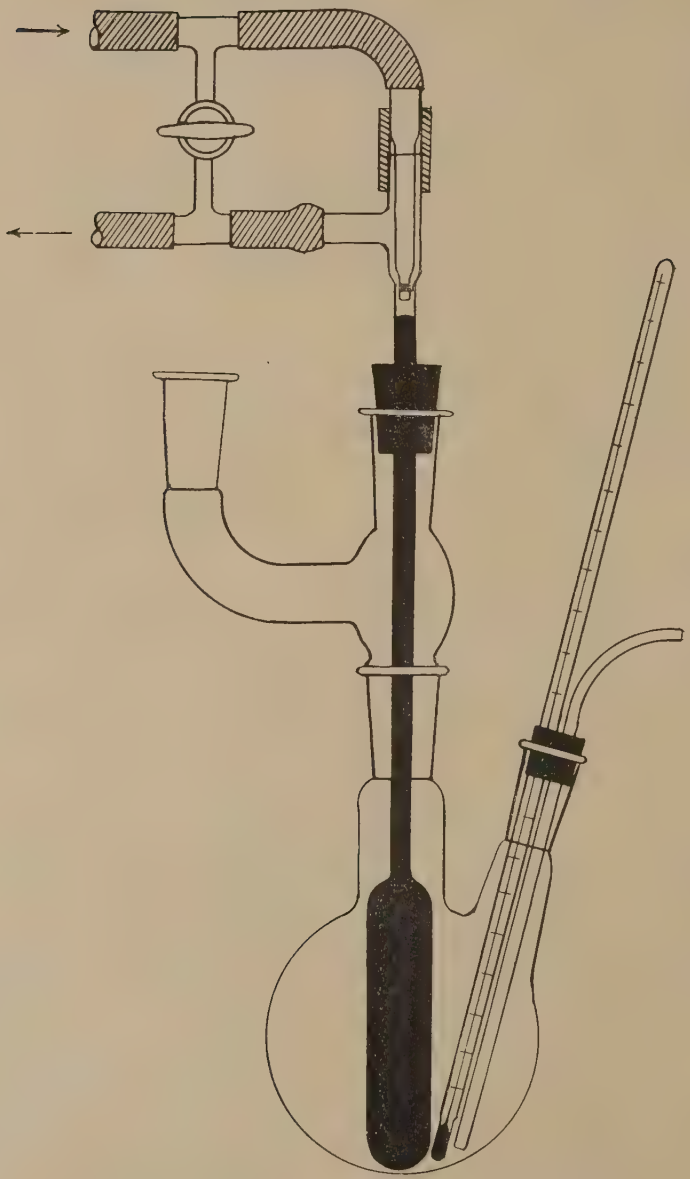
(7) The mitigation of fluorosis by management of flocks.

(8) A satisfactory explanation of anomalies in the incidence of fluorosis in sheep as judged from water analyses.

### Methods of Analysis.

The method of analysis used in these investigations is essentially that published in a report of a Sub-Committee of the Analytical Methods Committee (Society of Public Analysts, Great Britain, 1944).

Because of the large number of fluorine analyses required, some automatic system of temperature control was desirable in order to maintain a constant temperature at the required optimum (140°C.) in the distillation flask. The equipment devised for this purpose is shown on page 64. It consists essentially of a 250 ml. "Quickfit" flask with a sidearm carrying a steam inlet and a thermometer. A glass bulb and column filled with mercury fits almost to the base of the flask. Gas heating is used, the gas passing through a small jet centrally placed above the mercury column. The position of the jet is then adjusted so that at the desired temperature (140°C.) the mercury column rises to just cut off the flow of gas. A stopcock provides a bypass of gas to the burner, the position of the cock being adjusted to maintain a small flame when the thermostat takes control.



Automatic Temperature Control System in Fluorine Analysis.

Some difficulty was experienced in defining sources of fluorine contamination. This was traced to three causes:—

(1) The technique for the preparation of fluorine-free calcium hydroxide, as outlined by the above method, did not yield a fluorine-free product, even when A.R. grade chemicals were employed. The method outlined by MacIntyre (1945) was adopted and has proved satisfactory.

(2) The glass beads used in the distillation flask, both as a source of silica and to prevent bumping, were found to contain fluorine. The substitution of pyrex glass chips proved satisfactory.

(3) The electric furnace used in the ashing of samples was shown to be unsatisfactory. Apparently the lining of the muffle was either cemented with a fluoride flux or contaminated with fluorine from previous ignitions. The use of a non-cemented furnace eliminated this source of error.

A large number of water samples collected in connection with the Fluorine in Water Survey were analysed by the method of Clawson, Khalif and Perks (1940).

Samples were prepared for analysis as follows:—Soils were air dried, powdered and oven dried at 105°C.; pasture was air dried, ground in a Wiley mill, and dried in an air oven at 105°C.; biological material was dried in a vacuum oven prior to grinding in a Wiley mill; bones and teeth were thoroughly cleaned, dried, broken into small pieces, extracted with ether in a Soxhlet, after which bones were ground in a Wiley mill and teeth pulverised in a percussion mortar.

### Transmission Studies.

A survey of the available literature gives conflicting data on the fluorine content of milk. Phillips, Hart and Bohstedt (1934) noted that cows ingesting rock phosphate excreted fluorine in the milk at the rate of 0·25 to 0·5 p.p.m., which is comparable with amounts excreted by normal animals. On the other hand, Huffman (1938) stated that increased amounts of fluorine are excreted in the milk, but he did not quote levels. A Memorandum on Industrial Fluorosis near Fort William in Scotland (1949) published information on the fluorine levels in the milk of cows and ewes grazing on fluorine contaminated pasture. The highest figure for cows' milk was 0·44 p.p.m.F and for ewes' milk 0·62 p.p.m.F, levels which were not considered to be of practical importance.

Smith, Lantz and Smith (1935) found that children did not show mottling of the deciduous teeth where the mother was using water containing up to 6 p.p.m.F. The results of transmission studies with rats were in agreement with these findings. However, further investigation showed that severe mottling of deciduous teeth did occur when the mother used water

containing a high fluorine level (e.g., 12 p.p.m.F). These workers concluded that this indicated the passage of fluorine either into the foetal system or into the mother's milk.

The possibility of fluorine excretion in milk of cows depastured in endemic areas of Queensland and confined to artesian water containing 7 p.p.m.F was examined. The level of fluorine in the milk did not exceed 0·25 p.p.m.

It was decided to investigate the fluorine transmission both through foetal circulation and through the milk of ewes exposed to water containing fluorine as a soluble fluoride. The value of information on fluorine transmission, under conditions of exposure to fluorine in the drinking water such as obtain in endemic areas of Queensland, would lie in its bearing on stock management.

Six suitable ewes, of predominantly Merino breed and with lambs at foot, were selected. They were divided into three groups each of two animals. The ration was lucerne chaff *ad lib.* plus 4 oz. of maize meal daily. In all groups the composition of the drinking water was adjusted to correspond with that of an affected property—namely, 30 grains per gallon (430 p.p.m.) sodium chloride and 30 grains per gallon (430 p.p.m.) sodium bicarbonate —and the fluorine level adjusted in each group as under:—

Group 1 on water containing 2 p.p.m.F

Group 2 on water containing 5 p.p.m.F

Group 3 on water containing 10 p.p.m.F

At the end of the second week the following procedure was adopted. Ewes and lambs were separated, and the next morning each lamb was allowed access to the ewe for a few minutes. This made the ewe let down her milk and a sample was then taken by hand. Samples of milk from each ewe were collected at weekly intervals and analysed. Irrespective of the fluorine content of the water (up to 10 p.p.m.F), the level of fluorine in the milk did not exceed 0·2 p.p.m. for the 11 weeks during which the ewes remained in milk. Water consumption averaged 4·5 litres for each sheep daily.

Further studies on fluorine transmission by these experimental ewes were delayed until the next lambing for the following reasons:—

(1) The ewes had not been exposed to fluorided water prior to the first lambing.

(2) The possibility that lack of enhanced fluorine excretion might be due to fluorine storage.

(3) The assumption that by the next lambing the ewes' storage depots for fluorine would have reached saturation.

During the intervening period, the ewes were divided into two groups and fed lucerne *ad lib.* Group 1 was placed on water containing 2 p.p.m.F and Group 2 on water containing 10 p.p.m.

These two levels were selected because—

(1) They represent a "low" and a "high" fluorine intake as judged by clinical studies in affected areas of Queensland.

(2) Fluorine levels greater than 10 p.p.m. are not common in endemic areas of Queensland although concentration by water evaporation may allow an increase to a level in excess of 10 p.p.m. (50 p.p.m.F has been recorded in water stored in an earthen tank).

(3) The harmful effects to the unborn lamb by foetal circulation of fluorine had already been indicated by field observation where the fluorine level in the drinking water was 15 p.p.m.

(4) Fluorine levels in excess of 12 p.p.m.F were known to be transmitted by the parent to cause serious damage in human nutrition.

In addition to information on foetal transmission, some knowledge of the effect of a lowering of the ewe's calcium and protein intake and the possible result on fluorine excretion in the milk was desired. A ration high in protein and/or lime might conceivably exert a buffering effect and thus lessen the toxic action of fluorine and limit the excretion in milk. This is discussed further in the section on Dietary Mitigation Studies (page 74).

For the first two weeks after the second lambing, the ewes were kept on a ration of lucerne chaff. Again the fluorine content of the milk did not exceed 0·2 p.p.m.F. During the third week, oaten chaff was gradually introduced. The fluorine level increased to 0·3 p.p.m. in Group 1 and to 0·6 p.p.m. in Group 2. By the fourth week the ewes were on oaten chaff only and the fluorine level in the milk was back to 0·2 p.p.m. It did not again exceed this level. The protein and the calcium levels in the lucerne and oaten chaff were:—lucerne chaff, 16·1% protein and 0·73% lime (CaO); oaten chaff, 6·8% protein and 0·11% lime (CaO).

The lambs did not have access to the fluorided water available to the ewes. Each pen was fitted with a creep sufficiently low to permit entrance by the lamb but not by the ewe. The lambs were given fluorine-free water in the creep. Water tins were mounted on a raised platform sufficiently high to be out of reach of lambs but accessible to the ewes. Sloping barriers of plain galvanized iron prevented the lambs from climbing.

The lambs were slaughtered at six weeks of age and two lambs of approximately the same age and depastured on fluorine-free water were slaughtered as controls. Slaughter at this stage rather than at birth was for the following reasons:—

(1) It enabled the ewes to be kept in milk long enough to investigate—

(*a*) Fluorine excretion in milk by ewes exposed to fluorided water for a long period. (The possibility of lack of fluorine excretion in milk, due to storage in the first trial, was thus eliminated.)

(*b*) The effect of lowering both calcium and protein intake in the ration on fluorine excretion in the milk. (The possibility of lack of fluorine excretion in milk, due to a high protein and/or high calcium diet in the first trial, was thus eliminated.)

(2) The low fluorine content of the milk, as determined by analysis, indicated that this source of fluorine to the lambs would not affect deductions on foetal transmission.

(3) Analysis of the ration indicated that this source of fluorine would not affect deductions on foetal transmission.

(4) Weight gains in lambs during this period should not interfere, as fluorine accumulation in bones and teeth was to be compared with that in lambs of the same age, born from ewes not exposed to fluorided water.

Table 1 records the fluorine content of bones and teeth, and Table 2 the fluorine levels found in the organs of lambs from each group.

**Table 1.**

FLUORINE CONTENT OF BONES AND TEETH (P.P.M.).

| Bone or Teeth. | Control Group. | | | | Group 1. | | Group 2. | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Lamb 1. | | Lamb 2. | | Lamb 1. | | Lamb 1. | | Lamb 2. | |
| | Water-fat-free bone. | Ash. | Water-fat-free bone. | Ash. | Water-fat-free bone. | Ash. | Water-fat-free bone. | Ash. | Water-fat-free bone. | Ash. |
| Radius .. | 56 | 76 | 46 | 65 | 42 | 58 | 90 | 122 | 118 | 163 |
| Metacarpus | 45 | 59 | 34 | 48 | 48 | 67 | 107 | 133 | 93 | 128 |
| Femur .. | 42 | 59 | 66 | 97 | 30 | 47 | 80 | 117 | 109 | 152 |
| Tibia .. | 45 | 61 | 54 | 75 | 32 | 46 | 112 | 156 | 101 | 138 |
| Metatarsus | 45 | 57 | 40 | 55 | 26 | 36 | 85 | 113 | 104 | 129 |
| Mandible .. | 50 | 67 | 88 | 116 | 26 | 33 | 75 | 97 | 118 | 153 |
| Middle incisor .. | 32 | 40 | 27 | 31 | 43 | 55 | 85 | 102 | 117 | 141 |
| 1st molar | 37 | 47 | 43 | 51 | 53 | 69 | 59 | 74 | 69 | 86 |
| 4th rib .. | 48 | 70 | 66 | 98 | 29 | 48 | 59 | 83 | 96 | 133 |
| .. | 32 | 45 | 50 | 74 | 32 | 52 | 67 | 103 | 98 | 148 |

**Table 2.**

FLUORINE CONTENT OF ORGANS (P.P.M. DRY MATTER).

| Organ. | Control Group. | | Group 1. | Group 2. | |
|---|---|---|---|---|---|
| | Lamb 1. | Lamb 2. | Lamb 1. | Lamb 1. | Lamb 2. |
| Liver .. | 1·0 | 0·6 | 0·7 | 1·3 | 1·0 |
| Kidney .. | 4·5 | 2·3 | 2·2 | 6·0 | 7·3 |
| Heart .. | 2·2 | 3·1 | 2·7 | 4·0 | 1·8 |
| Spleen .. | 2·2 | 2·2 | 1·7 | 0·8 | 1·7 |

The lamb from group 1 shows no significant increase in the fluorine content of any bone or organ. Lambs from group 2 show a definite increase in fluorine in all bones, indicating that some fluorine is transmitted to the foetus by ewes on water containing 10 p.p.m.F. No abnormalities were to be seen in bones and teeth from lambs in this group. The level of fluorine in bones and teeth of lambs in group 2, although much greater than that in group 1, was still only about one-half of that to be found in adult sheep not exposed to fluorine in the drinking water. It would therefore be reasonable to assume that the fluorine transmitted through foetal circulation, where ewes are maintained solely on water containing up to 10 p.p.m.F, is too small to have results harmful to the lamb.

The following conclusions may be drawn from these studies:—

(1) Ewes on water containing up to 10 p.p.m.F, and irrespective of the quality of pasture or ration, do not excrete an increased amount of fluorine in the milk.

(2) Ewes on water containing 10 p.p.m.F transmit fluorine to the foetus, but it is believed that the amount is too small to constitute a danger to the lamb. At higher levels of fluorine intake this transmission to the foetus may be deleterious to the lamb. Field evidence indicates damage to the unborn lamb when ewes are maintained on water containing 15 p.p.m.F. Fluorine levels in excess of 10 p.p.m. are not common in the endemic areas of this State.

These results have the following significance in stock management:—

(1) On water containing up to 10 p.p.m.F it is possible to breed with safety to the lamb. This practice would not be recommended for young breeding ewes (up to two years), as a fluorine level of 10 p.p.m. would be harmful to the ewe.

(2) As no appreciable amount of fluorine is transmitted through the milk, there is no need to wean early. The removal of ewe and lamb to fluorine-free water at 6 to 8 weeks is advisable because at this age lambs begin to drink appreciable quantities of water. After weaning, lambs should be held either on surface water or on bore water containing less than 2 p.p.m.F.

**Alternative Sources of Fluorine to Sheep in Endemic Areas.**

Field observations on a number of properties have failed to show any degree of correlation between the incidence of the disease and the fluorine content of the water. Various factors, such as an alternative fluorine-free water supply (surface water), the seasonal conditions prevailing and the type of management practised, may be responsible for this. The effect of evaporation on the fluorine concentration has also to be considered where stock are watering at some distance from the borehead. The possibility that there is some factor in the pasture which delays or mitigates the onset of symptoms is discussed in the section on Dietary Mitigation Studies (page 74).

A further possibility is that there is another source of fluorine intake apart from the water. There is no information available on the uptake of fluorine by pasture plants or the accumulation of fluorine in soils which have had contact with fluorided water either by flooding or by channelling in endemic areas of Queensland.

An examination was made of pasture growing along bore drains and on areas flooded by breaks in such drains, of soil samples from all areas in which pasture was collected, and of the bore water at the points at which such collections were made. The importance of these studies lies in the fact that, in rainless seasons, this pasture or "green pick" along an extensive bore drain reticulating system supports many sheep.

Table 3 records the analysis of water, soil and pasture.

**Table 3.**

Analyses of Water, Soil and Pasture from an Endemic Area.

| Where taken. | Water. | Soil. | | Pasture. | | | |
|---|---|---|---|---|---|---|---|
| | p.p.m. F. | p.p.m. F in dry matter. | pH. | Type. | p.p.m. F in dry matter. | Crude Protein. | Lime (CaO). |
| | | | | | | % | % |
| At borehead | 0·5 | | | | | | |
| 3½ miles along the bore drain from borehead | 1·5 | | | | | | |
| At terminus after 5 miles along drain from borehead | 1·9 | 18·0 | 8·5 | Couch grass (*Cynodon dactylon*) | 0·5 | 7·4 | 0·69 |
| After 3 miles along another drain from same borehead | 1·6 | 11·2 | 8·2 | Paspalum (*Paspalum dilatatum*) | 0·5 | 4·5 | — |
| | | | | Couch grass (*Cynodon dactylon*) | 0·5 | 7·1 | 0·73 |
| | | | | Channel grass (*Diplachne muelleri*) | 1·0 | 4·9 | 0·62 |
| At borehead | 3·6 | | | | | | |
| 3 miles along bore drain from borehead | 3·7 | 20·0 | 9·0 | Couch grass (*Cynodon dactylon*) | 0·5 | 12·3 | 0·22 |
| 10 miles along bore drain from borehead | 7·2 | 31·0 | 8·9 | Couch grass (*Cynodon dactylon*) | 1·0 | 6·0 | 0·85 |
| 13 miles along bore drain from borehead | 10·5 | 43·0 | 10·0 | Couch grass (*Cynodon dactylon*) | 1·0 | 5·4 | 0·64 |
| (Check soil sample 20 yd. from drain) | | 13·0 | 8·1 | | | | |

**Table 3.**—*continued.*

ANALYSIS OF WATER, SOIL AND PASTURE FROM AN ENDEMIC AREA—*continued.*

| Where taken. | Water. | Soil. | | Pasture. | | | |
|---|---|---|---|---|---|---|---|
| | p.p.m. F. | p.p.m. F. in dry matter. | pH. | Type. | p.p.m. F. in dry matter. | Crude Protein. | Lime (CaO). |
| | | | | | | % | % |
| At borehead | 1·3 | | | | | | |
| 8 miles along bore drain from borehead | 3·3 | 7·0 | 10·2 | Channel grass (*Diplachne muelleri*) | 13·5 | — | — |
| | | | | Red burr (*Bassia echinopsila*) | 10·0 | — | — |
| | | | | Couch grass (*Cynodon dactylon*) | 7·0 | 6·0 | 0·77 |
| | | | | Saltbush (*Atriplex* sp.) | 11·0 | 12·0 | 1·29 |
| | | | | Pigweed (*Portulaca oleracea*) | 3·0 | 7·7 | 1·56 |
| 14 miles along bore drain from borehead | 5·5 | 11·0 | 9·4 | Couch grass (*Cynodon dactylon*) | 11·0 | 7·8 | 0·53 |
| | | | | Mitchell grass (*Astrebla* sp.) | 2·0 | 5·5 | 0·44 |
| At borehead | 6·5 | | | | | | |
| 2 miles along bore drain from borehead | 12·5 | 38·0 | 8·5 | Couch grass (*Cynodon dactylon*) | 1·0 | 6·4 | 0·67 |
| At terminus 4 miles along drain from borehead | 32·0 | 35·0 | 9·4 | Couch grass (*Cynodon dactylon*) | 15·0 | 14·9 | 0·76 |
| (Check soil sample 20 yd. from drain) | .. | 13·0 | 8·5 | | | | |
| At borehead | 7·5 | | | | | | |
| 3 miles along bore drain from borehead | 10·5 | 30·0 | 8·5 | Couch grass (*Cynodon dactylon*) | 5·5 | 7·3 | 0·65 |
| | | | | Button grass (*Dactyloctenium radulans*) | 3·0 | 10·0 | 0·72 |
| At terminus 6 miles along drain from borehead | 25·0 | 23·6 | 8·4 | Channel grass (*Diplachne muelleri*) | 9·0 | 10·7 | 0·32 |
| | | | | Saltbush (*Atriplex* sp.) | 40·0 | 9·4 | 1·22 |

**Table 3.**—*continued.*

ANALYSIS OF WATER, SOIL AND PASTURE FROM AN ENDEMIC AREA—*continued.*

| Where taken. | Water. | Soil. | | Pasture. | | | |
|---|---|---|---|---|---|---|---|
| | p.p.m. F. | p.p.m. F. in dry matter. | pH. | Type. | p.p.m. F. in dry matter | Crude Protein. | Lime (CaO). |
| At borehead | 2·5 | | | | | | |
| 3 miles along bore drain from borehead | 2·7 | 22·0 | 8·8 | Couch grass (*Cynodon dactylon*) | 1·0 | 9·9 | 0·87 |
| At terminus 6 miles along drain from borehead | 6·0 | 20·0 | 8·6 | Couch grass (*Cynodon dactylon*) | 2·0 | 6·0 | 0·60 |
| At borehead | 5·1 | | | | | | |
| 3 miles along bore drain from borehead | 6·2 | 29·6 | 8·7 | Couch grass (*Cynodon dactylon*) | 1·0 | 6·7 | 1·02 |
| | | | | Mitchell grass (*Astrebla* sp.) | 0·5 | 8·8 | 0·64 |
| At terminus 6 miles along drain from borehead | 8·0 | 38·6 | 9·2 | Channel grass (*Diplachne muelleri*) | 0·5 | 12·0 | 0·31 |
| (Check soil sample 20 yd. from drain) | | 14·0 | 8·3 | | | | |
| At borehead | 3·3 | | | | | | |
| 6 miles along bore drain from borehead | 4·8 | 22·6 | 8·0 | Star grass (*Chloris* sp.) | 1·0 | 11·3 | 0·45 |
| | | | | Couch grass (*Cynodon dactylon*) | 1·0 | 10·2 | 0·75 |
| | | | | Button grass (*Dactyloctenium radulans*) | 0·5 | 7·5 | 0·55 |

The following conclusions may be drawn from this investigation:—

*Water.*—The water analyses show the significance of evaporation with distance down the bore drain. The importance of this factor in property management is apparent. Even higher fluorine levels must be expected in terminal dams or tanks where there is a wide surface to volume ratio. This is particularly true of the more arid areas where the effective evaporative loss may reach 100 inches annually. One case was encountered where a water sample, taken from a bore drain at a point several miles from the borehead, contained less fluorine than water at the borehead. Further investigation showed that deposits of "kopi" (calcium sulphate) were to be found along

parts of the bore drain and that this was responsible for removal of fluorine from the water. This has been confirmed in other areas where fluorided bore water is reticulated through "kopi" deposits. The application of this finding in the purification of fluorided water will be discussed later.

*Soils.*—While in most cases there is an increase in the level of flourine in the soil with increase in the distance from the borehead, there is no relationship between fluorine in the soil and fluorine in the plant.*

It would appear that, in some of the alkaline soils of western Queensland, fluorine as sodium fluoride is converted to an insoluble fluoride and is not readily available to the plant. No evidence of earth eating by stock was seen in the areas visited, but this should not be excluded as an alternative source of fluorine intake in other areas or adventitious intake during drought.

*Pasture.*—The data show that some plants growing either along bore drains reticulating fluorided water or on land flooded by breaks from these drains can build up a dangerous level of fluorine. Observations in western Queensland have shown that the water consumption of a grown sheep does not exceed five gallons per week, while its food consumption may exceed 3 lb. of dry matter daily. Hence, as a guide, it may be assumed that the animal's daily intake of dry matter is approximately half the weight of water drunk. Consequently, plants containing 4 p.p.m.F must be viewed with suspicion, as the fluorine they supply is comparable with that of water containing 2 p.p.m.F. The possibility that this may need modification in view of the high lime and/or protein content in many western Queensland pasture plants is discussed in the next section.

* The question of fluorine uptake by plants was also investigated in a locality close to Brisbane. This area is almost exclusively devoted to the production of fruit and vegetables, and very heavy application of fertilizer, in particular superphosphate, is widely practised. Superphosphate contains up to 1% of fluorine and a high percentage of calcium sulphate. The fluorine levels found in soils and in vegetables are as follows:—

| Crop. | Moisture in Crop. | Fluorine in Crop (moisture-free). | Fluorine in Soil (air-dried). |
|---|---|---|---|
| | % | p.p.m. | p.p.m. |
| Cabbage plants (from seed-bed) | 89·3 | 3·0 | 48·0 |
| Tomatoes .. .. .. | 93·8 | 0·4 | 25·0 |
| Tomatoes .. .. .. | 93·8 | Nil | 30·0 |
| Beans .. .. .. .. | 92·5 | 0·4 | 25·0 |
| Marrow .. .. .. .. | 97·3 | Nil | 44·0 |
| Potatoes .. .. .. .. | 85·6 | Nil | 34·0 |
| Cabbage .. .. .. .. | 93·5 | 0·4 | 19·0 |

There is no appreciable uptake of fluorine by vegetables grown in soils heavily fertilized with superphosphate containing 1% fluorine. Either these plants do not abstract fluorine or the fluorine in the soil is present in a form which is not available to the plant. In view of the high level of calcium sulphate in superphosphate, the formation of an insoluble calcium fluoride in the soil would be anticipated.

## Dietary Mitigation Studies.

The prevalence of fluorosis in Queensland is such that any economically sound measure likely to reduce its incidence merits serious consideration.

It has been reported that an insufficient supply or an imbalance of other inorganic constituents of the feed has been observed to influence the toxicity of ingested fluorine, while the addition of extra calcium phosphate to the ration has been reported to reduce the toxicity of calcium fluoride for sheep (Velu, 1933). Peirce (1939) stated that the toxic effect of fluorine is seemingly enhanced in poorly nourished sheep.

A number of experiments with small animals have been recorded. Smith and Shaner (1944) found that, if given with calcium carbonate or magnesium oxide, a lethal dose of sodium fluoride can be administered to guinea pigs without harmful effects. Irving (1946), working on rats, found that when the blood calcium is raised the action of fluorine on the predentin is greatly reduced or even prevented, but when it is lowered the effects of fluorine are enhanced. Ranganathan (1944), also working on rats, claimed that the lactate, gluconate, phosphate, oxide, carbonate and chloride of calcium all have the same potency in mitigating the toxic effect of fluorine; the salts of magnesium also offer some protection, and those of aluminium and barium offer none. He also claimed that the toxicity of fluorides of calcium, magnesium and sodium cannot be correlated with their solubilities; magnesium fluoride, which is the least soluble, is the most toxic. Pillai, Rajagopalan and De (1944) found that whole milk powder very largely protected the teeth of rats against mottling, and that whole fresh egg or fish powder, which included fish bones, delayed but did not prevent the development of mottled teeth and generalised fluorosis. Half a gram of bone powder was also beneficial.

Greenwood *et al.* (1946) found that, at the same level of fluorine intake, sodium fluoride produced dental fluorosis and a high storage of fluorine in the bones of dogs; calcium phosphate ($Ca_3(PO_4)_2$) gave dental hypoplasia; and bone meal and defluorophos (fluorine-free calcium acid phosphate, a variation of bone) gave well-formed teeth and a minimum of staining, while fluorine storage in the bones was not excessive.

Majundar and Ray (1946), using sodium fluoride as a source of fluorine, carried out metabolism studies on hill bulls. They concluded that fluorosis is rapidly produced in these animals by combining a high intake of fluorine with a low intake of phosphorus or with a wide Ca : P ratio, but that when the Ca : P intake is adjusted to an optimum ratio and fed in adequate quantities the onset of fluorosis is delayed. Estimation of the net retention of fluorine

gave no indication of the condition of the animal, but the poorer the condition the greater the quantity of fluorine excreted in the urine. Hence urinary output of fluorine is a good index of the intensity of fluorine intoxication. These workers also claimed that the ingestion of aluminium sulphate is effective in preventing fluorosis.

The treatments suggested by these studies are not immediately applicable to the control of endemic fluorosis in sheep in Queensland, firstly because in no case was fluorine ingested in solution, and secondly because without further evidence the data cannot be held to be applicable to sheep.

An investigation of the onset of fluorosis in Merino sheep on diets containing various combinations of calcium, phosphate and protein was designed. It was not possible to use large numbers of sheep in the experiment or to simulate field grazing conditions, particularly the dry-season stubble-like pasture which aggravates the onset of dental lesions. Further, it was expected that lack of exercise, different climatic conditions and the use of chaffed feed would reduce the water consumption normal in endemic areas. It was anticipated, however, that gross differences between groups would be apparent if any treatment offered some protection against fluorosis.

A low-lime basal ration of oaten chaff was fed in most cases and fluorine was supplied as the sodium salt in solution in the drinking water at levels of 5 and 10 p.p.m., representing "bad" and "very bad" field conditions. Simultaneously, an attempt to delay the onset of the disease was made by adding to the oaten chaff ration (1) a calcium supplement (using powdered limestone, calcium sulphate or sterilized bone-meal); (2) a protein supplement (using peanut meal); or (3) a calcium-protein supplement (using peanut meal plus powdered limestone). An additional group was fed on lucerne chaff only.

The calcium supplements were selected on the scores of availability, cheapness and ease of administration. It is possible to feed them either as a lick or, with slight variations, through the water. For example, calcium carbonate (limestone) may be fed as a lick or placed in the water as bicarbonate or hydroxide. Calcium sulphate may be fed as such or used in the water. Fluorine-free calcium acid phosphate (a variation of bone), though not used in this experiment, might be considered as an alternative treatment of fluorided water.

The buffering of protein and protein plus lime, in the form of peanut meal and lucerne, was investigated on the assumption that lambs after weaning, and in the period between wet seasons (and hence surface water), might be fed for short intervals on a protein buffer without exceeding the limits imposed by finance.

Details of the rations are given hereunder:—

| Group. | Daily Ration per Sheep. | Composition of Ration. | | |
|---|---|---|---|---|
| | | CaO. | $P_2O_5$. | Protein. |
| | | % | % | % |
| 1 | 1 lb. oaten chaff .. .. .. .. | 0·2 | 0·2 | 5–7 |
| 2 | 1 lb. oaten chaff plus 4·5 grams powdered limestone .. .. .. .. .. | 0·8 | 0·2 | 5–7 |
| 3 | 1 lb. oaten chaff plus 6·75 grams bonemeal | 0·8 | 0·6 | 5–7 |
| 4 | 1 lb. oaten chaff plus calcium sulphate (saturated solution) in drinking water | 0·6–1·0 | 0·2 | 5–7 |
| 5 | 1 lb. oaten chaff plus 4 oz. peanut meal .. | 0·2 | 0·4 | 14·0 |
| 6 | 1 lb. oaten chaff plus 4 oz. peanut meal plus 4·25 grams powdered limestone .. .. | 0·8 | 0·4 | 14·0 |
| 7 | 1 lb. lucerne chaff .. .. .. .. | 0·8 | 0·5 | 16·0 |

(A standard source of vitamins A and D was fed to animals in Groups 1–6.)

Each group consisted of 8 four-month-old Merino lambs, and was divided into sub-groups of four animals each, sub-group A receiving water containing 5 p.p.m.F and sub-group B water containing 10 p.p.m.F. The mineral composition of these waters was made comparable with that found in endemic areas—i.e., they contained 30 grains per gallon (430 p.p.m.) of sodium bicarbonate and 30 grains per gallon (430 p.p.m.) of sodium chloride.

A 44-gallon drum, complete with tap, was set up in each pen. In the preparation of each 44 gallons of water, very thorough mixing was essential to ensure a uniform fluorine level of either 5 or 10 p.p.m. This water was analysed at regular intervals. For pens 4A and 4B an excess of calcium sulphate was added when the waters were prepared, and at least 24 hours were allowed for settling. The position of the tap (2-3 inches from the base of the drum) prevented the excess of calcium sulphate from running into the drinking trough. It was soon found that it was not possible to maintain a level of 10 p.p.m.F in group 4B. Presumably the relatively insoluble calcium fluoride was precipitated, and this precipitation appeared to be much quicker in the case iron drum than in glass bottles in the laboratory. This feature will be discussed later. Sub-group 4A was adjusted to 5 p.p.m.F and sub-group 4B varied from 7 to 5 p.p.m.F.

The quantities fed were determined on the composition of the rations, as shown. Lucerne chaff was taken as the working standard, and the levels of lime and protein added to the basal ration were adjusted to this standard. To ensure palatability, the limestone and bonemeal were mixed with an equal volume of icing sugar (a finely powdered cane sugar containing about 5% of wheaten flour).

Before the experiment commenced in April 1947, the sheep were shorn, treated for internal parasites, and weighed. Weighing was repeated monthly. The weight variations for each group are shown in Fig. 7.

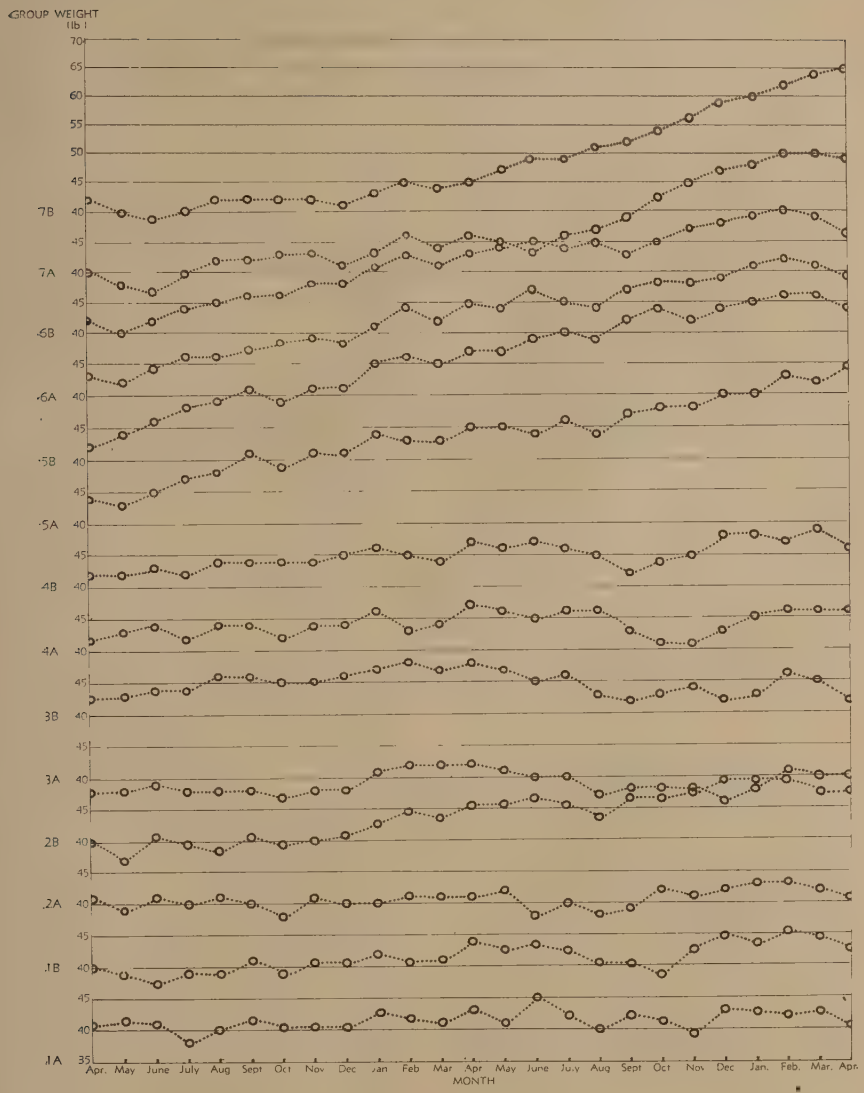



Fig. 7.
Weight Variations of Groups.

Table 4 records the onset of symptoms of fluorosis in each experimental group as judged by monthly examinations of the permanent incisor teeth.

**Table 4.**

MONTHLY EXAMINATION OF INCISOR TEETH.*

*Group* 1.

| Month. | Sub-Group 1A. | Sub-Group 1B. |
|---|---|---|
| 1947. | | |
| May | Normal temporary incisors. | Normal temporary incisors. |
| June | ,, ,, ,, | ,, ,, ,, |
| July | ,, ,, ,, | ,, ,, ,, |
| Aug. | ,, ,, ,, | ,, ,, ,, |
| Sept. | 1st permanent incisor erupted in one sheep. | 1st permanent incisor erupted in one sheep. |
| Oct. | 1st permanent incisor half up in one sheep and shows some erosion. | Incisor half up and shows surface erosion. |
| Nov. | 1st permanent incisors erupted in all sheep; they have white appearance and show erosion. | Incisor erupted in second sheep. |
| Dec. | 1st pair of incisors well up, paper white and eroded. | Incisors erupted third sheep; all show eroded surfaces and very white appearance. |
| 1948. | | |
| Jan. | 1st pair in wear, erosion more pronounced. | No change. |
| Feb. | No change. | No change. |
| Mar. | No change | All 1st pair of incisors erupted; show considerable erosion, slight chipping and slight staining. |
| Apr. | 1st pair markedly eroded and show some chipping of the cutting edges. | No change. |
| May | Chipping more pronounced. | No change. |
| June | 2nd pair of incisors erupting in one sheep. | 1st pair show bands of chalkiness at the base, considerable erosion and some chalky areas. One sheep erupting 2nd pair of incisors, banding more marked. |
| July | 1st pair show tendency to banding at base. | No change. |
| Aug. | 2nd pair half up on two sheep, eroded, and show chalky patches. | 2nd pair in two sheep, chalky, eroded and banded. |
| Sept. | 2nd pair badly eroded, paper white and banded over whole surface. | 2nd pair in three sheep. |
| Oct. | No change. | Banding very noticeable in all incisors. |
| Nov. | 2nd pair show some chipping. | No change. |
| Dec. | No change. | Some chipping of 1st pair. |
| 1949. | | |
| Jan. | No change. | All teeth show marked erosion, striations and chalky areas. |
| Feb. | All teeth show erosion, chalky areas, and some chipping. | No change. |
| Mar. | No change. | No change. |
| Apr. | Fine striations on all teeth. | All teeth very white, eroded, and show fine traverse striations. |

* *Nomenclature.*—Temporary incisors—the deciduous incisor teeth. Permanent incisors—1st pair erupting in normal sheep at 12-18 months, 2nd pair at 18-24 months, 3rd pair at 30-36 months, 4th pair at 42-48 months.

*Group* 2.

| Month. | Sub-Group 2A. | Sub-Group 2B. |
|---|---|---|
| 1947. | | |
| May | Normal temporary incisors. | Normal temporary incisors. |
| June | ,, ,, ,, | ,, ,, ,, |
| July | ,, ,, ,, | ,, ,, ,, |
| Aug. | ,, ,, ,, | ,, ,, ,, |
| Sept. | 1st permanent incisor erupted in one sheep. | ,, ,, ,, |
| Oct. | Permanent incisors erupted in three sheep; all show erosion; 1st pair overlap in one sheep. | 1st permanent incisors erupted in one sheep. |
| Nov. | Incisors paper white and markedly eroded. | Incisors in one sheep only and show erosion. |
| Dec. | No change. | Incisors in two sheep. |
| 1948. | | |
| Jan. | No change. | Incisors erupted in all sheep; all show erosion. |
| Feb. | 1st pair erupted in all sheep. | 1st pair overlap badly in one sheep. |
| Mar. | Bands of chalkiness over lower portion of 1st pair, all eroded and paper white. | Pronounced banding of all first incisors, all paper white. |
| Apr. | Erupting 2nd pair in one sheep. | No change. |
| May | Erupting 2nd pair in two sheep. | No change. |
| June | 2nd pair show chalky areas. | 1st pair show deep indentations on labial surface. |
| July | No change. | No change |
| Aug. | 2nd pair show striations, 1st pair starting to chip. | Erupting 2nd pair, badly deformed in one sheep. |
| Sept. | No change. | No change. |
| Oct. | Bands of chalkiness plus severe erosion over the whole surface of the 2nd pair and slight chipping. | Bands of chalkiness plus severe erosion over the whole surface of the 2nd pair and slight chipping. |
| Nov. | No change. | No change. |
| Dec. | Chipping more pronounced. | No change. |
| 1949. | | |
| Jan. | No change. | 3rd pair erupting in one sheep. |
| Feb. | Pronounced erosion in all permanent teeth. | No change. |
| Mar. | No change. | Pronounced erosion and marked striations in all permanent teeth; one of the third pair erupted at right angles. |
| Apr. | Erosion, pitting and banding in all permanent teeth. | Erosion, pitting and banding more pronounced than in Group 2A. |

*Group* 3.

| Month. | Sub-Group 3A. | Sub-Group 3B. |
|---|---|---|
| 1947. | | |
| May | Normal temporary incisors. | Normal temporary incisors. |
| June | ,, ,, ,, | Erupting 1st pair of permanent incisors in one sheep. |
| July | ,, ,, ,, | No change. |
| Aug. | ,, ,, ,, | 1st pair half up in one sheep. |
| Sept. | ,, ,, ,, | No change. |
| Oct. | ,, ,, ,, | ,, ,, |
| Nov. | 1st pair of permanent incisors in one sheep. | 1st pair erupted in two sheep; 2nd pair erupting in one sheep. |

*Group 3—continued.*

| Month. | Sub-Group 3A. | Sub-Group 3B. |
|---|---|---|
| 1947. | | |
| Dec. | 1st pair erupted in two sheep, surfaces eroded. | All 1st incisors erupted and show some erosion; one sheep has a badly undershot jaw. |
| 1948. | | |
| Jan. | 1st pair erupted in all sheep. | Incisors show considerable erosion and bands of chalkiness at the base. |
| Feb. | All incisors show erosion, some chalky spots and banding at base of teeth. | All incisors markedly eroded, show some chalky spots, bands of chalkiness at the base and slight chipping. |
| Mar. | No change. | No change. |
| Apr. | ,, ,, | 2nd pair in one sheep, show chalky areas and banding. |
| May | Some chipping of cutting edges. | Some chipping of cutting edges. |
| June | Erupting 2nd pair in one sheep. | Erupted 2nd pair on three sheep, chalky striations most marked on all 1st incisors. |
| July | No change. | 2nd pair in one sheep erupted at right angles. |
| Aug. | Pronounced chipping of 1st pair; eroded and show bands of chalkiness. | 2nd pair eroded and markedly banded over whole surface. |
| Sept. | No change. | No change. |
| Oct. | ,, ,, | ,, ,, |
| Nov. | ,, ,, | Chipping more pronounced, chiefly in sheep with undershot jaws. |
| Dec. | Bands of chalkiness in all permanent teeth. | No change. |
| 1949. | | |
| Jan. | Striations more pronounced as 2nd pair come into wear. | Very pronounced striations at base in 1st pair and over the whole surface of 2nd pair. |
| Feb. | Some chipping of 2nd pair. | Some chipping of 2nd pair. |
| Mar. | No change. | No change. |
| Apr. | All permanent teeth paper white, badly eroded and striated with some chipping. | All permanent teeth paper white with chalky spots, badly eroded and striated, pronounced chipping and one badly deformed incisor arch due to crowding and abnormal position of individual teeth. |

*Group 4.*

| Month. | Sub-Group 4A. | Sub-Group 4B. |
|---|---|---|
| 1947. | | |
| May | Normal temporary incisors. | Normal temporary incisors. |
| June | ,, ,, ,, | ,, ,, ,, |
| July | ,, ,, ,, | Erupting 1st permanent incisor in one sheep. |
| Aug. | ,, ,, ,, | No change. |
| Sept. | ,, ,, ,, | Erupted 1st pair in three sheep; badly splayed in one sheep; roughened surfaces. |
| Oct. | ,, ,, ,, | All 1st incisors erupted and show erosion |

*Group 4—continued.*

| Month. | Sub-Group 4A. | Sub-Group 4B. |
|---|---|---|
| 1947, | | |
| Nov. | Erupting 1st pair of permanent incisors in three sheep. | All permanent teeth paper white and show general pitting over whole surface. |
| Dec. | All first incisors show roughened surfaces. | No change. |
| 1948. | | |
| Jan. | 1st pair paper white, show generalised pitting and tendency to chalkiness and bands. | ,, ,, |
| Feb. | 1st pair splayed in one sheep. | Bands of chalkiness at the base of 1st pair give a striated effect. |
| Mar. | No change. | 2nd pair erupting at right angles in one sheep. |
| Apr. | Slight chipping of 1st pair. | Marked indentations and some chalky areas on 1st incisors. |
| May | Chipping more pronounced. | 2nd pair erupted in two sheep and badly pitted, 1st pair chipped. |
| June | Erupting 2nd pair, at right angles in one sheep. | All 2nd pairs of incisors well up, chalky and badly eroded. |
| July | 2nd pair chalky and badly pitted. | No change. |
| Aug. | No change. | ,, ,, |
| Sept. | 1st pair show chalky bands at base and 2nd pair show similar bands over the whole surface. | Similar to 4A but chipping of 1st pair more marked. |
| Oct. | Banding more marked. | Marked banding on all permanent teeth. |
| Nov. | No change. | No change. |
| Dec. | All teeth very chalky with pronounced banding. | Erupting 3rd pair in one sheep. |
| 1949. | | |
| Jan. | No change. | No change. |
| Feb. | Pitting of 2nd pair more pronounced. | Striations on base of 1st pair and extend over whole surface of 2nd pair ; marked chipping. |
| Mar. | Chipping of 1st and 2nd pairs. | Advanced pitting in all incisors. |
| Apr. | All incisors paper white, eroded. striated and chipped ; one deformed incisor arch. | All teeth paper white. heavily eroded and striated with bad chipping ; one badly deformed incisor arch. |

*Group 5.*

| Month. | Sub-Group 5A. | Sub-Group 5B. |
|---|---|---|
| 1947. | | |
| May | Normal temporary incisors. | Normal temporary incisors. |
| June | ,, ,, ,, | ,, ,, ,, |
| July | ,, ,, ,, | ,, ,, ,, |
| Aug. | ,, ,, ,, | ,, ,, ,, |
| Sept. | Erupting 1st permanent incisors in two sheep. | ,, ,, ,, |
| Oct. | Permanent incisors in three sheep show roughened surface. | Erupting 1st permanent incisor in one sheep. |
| Nov. | Incisors in three sheep show erosion. | 1st pair of incisors erupting in two sheep. |
| Dec. | No change. | Incisors show erosion. |
| 1948. | | |
| Jan. | All 1st incisors erupted and show erosion. | All 1st incisors erupted and showing erosion. |
| Feb. | Erupting 2nd pair in one sheep. | No change. |

*Group 5—continued.*

| Month. | Sub-Group 5A. | Sub-Group 5B. |
|---|---|---|
| Mar. | Pitting on all incisors emphasised by slight staining. | Pitting on all incisors and emphasised by slight staining. |
| Apr. | Chipping of cutting surfaces. | General pitting and staining and some chipping. |
| May | 2nd pair show considerable pitting and striations; one has erupted at right angles. | 2nd pair show striations. |
| June | Chalky areas on all permanent teeth. | Some deep pitting and chalky areas. |
| July | No change. | Very difficult eruption in one sheep; also one 2nd incisor erupted at right angles. |
| Aug. | 1st pair show striations at the base, 2nd pair show erosion and banding over whole surface. | 1st pair show striations at the base, considerable erosion and chalky spots; 2nd pair show more damage. |
| Sept. | 2nd pair show considerable damage. | No change. |
| Oct. | Banding more pronounced as 2nd pair develop. | Very marked banding of 2nd pair. |
| Nov. | No change. | No change. |
| Dec. | Slight chipping of 2nd pair. | Slight chipping of 2nd pair. |
| 1949. | | |
| Jan. | All teeth are paper white, pitted and this emphasised by some staining; show chalky bands. | Irregular eruption of 2nd pair in three sheep, considerable pitting and banding. |
| Feb. | Erupting 3rd pair in one sheep. | Erupting 3rd pair in one sheep. |
| Mar. | 3rd pair paper white, considerably eroded and banded. | As for sub-group 5A. |
| Apr. | All incisors paper white, eroded, striated, one deformed incisor arch. | Damage greater than in 5A, and bad chipping. |

*Group 6.*

| Month. | Sub-Group 6A. | Sub-Group 6B. |
|---|---|---|
| 1947. | | |
| May | Normal temporary incisors. | Normal temporary incisors. |
| June | ,, ,, ,, | ,, ,, ,, |
| July | 1st permanent incisors erupting in one sheep. | ,, ,, ,, |
| Aug. | 1st pair erupting in two sheep. | ,, ,, ,, |
| Sept. | 1st pair erupted in three sheep, badly splayed in one and surfaces roughened. | 1st permanent incisors erupting in one sheep. |
| Oct. | 1st pair show erosion. | 1st pair erupting in two sheep. |
| Nov. | 1st pair show considerable erosion. | 1st pair erupted in three sheep and show erosion. |
| Dec. | All 1st permanent incisors erupted, show pitting and slight staining. | All 1st permanent incisors erupted and show pitting emphasised by staining. |
| 1948. | | |
| Jan. | No change. | Difficult eruption in one sheep. |
| Feb. | 1st pair show slight chipping, some chalky spots, erosion and slight staining. | 1st pair show general pitting and staining. |
| Mar. | 2nd pair erupting in one sheep. | 2nd pair erupting in one sheep, still a very difficult eruption of 1st pair in one sheep. |
| Apr. | Chalky spots on 1st pair and banding noticeable at base of teeth. | Some chalky areas and banding at base of teeth. |

*Group 6—continued.*

| Month. | Sub-Group 6A. | Sub-Group 6B. |
|---|---|---|
| 1947. | | |
| May | Chipping of cutting surfaces more pronounced. | No change. |
| June | 2nd pair erupted in two sheep, pitted and stained. | 2nd pair erupted in two sheep; chalky and heavily pitted. |
| July | Banding noticeable at base of 1st pair. | 1st pair banded at the base, 2nd pair show striations over whole surface. |
| Aug. | Bad chipping of 1st pair. | No change. |
| Sept. | 2nd pair almost in wear, eroded, banded and stained. | 2nd pair badly pitted and stained. |
| Oct. | No change. | No change. |
| Nov. | ,, ,, | 1st pair badly chipped. |
| Dec. | 3rd pair erupting in one sheep. | 3rd pair erupting in one sheep. |
| 1949. | | |
| Jan. | Marked striations on all incisors. | Marked striations on all incisors. |
| Feb. | 3rd pair erupting in two sheep; 2nd pair show chalky areas and bad chipping. | No change. |
| Mar. | Striations on permanent incisors. | Erosion and striations intensified from 1st to 2nd to 3rd pairs. |
| Apr. | All incisors paper white, show some chalky areas, marked erosion emphasised by slight staining and bad chipping. | All incisors heavily eroded, striated, stained and chipped; one badly deformed incisor arch. |

*Group 7.*

| Month. | Sub-Group 7A. | Sub-Group 7B. |
|---|---|---|
| 1947. | | |
| May | Normal temporary incisors. | Normal temporary incisors. |
| June | ,, ,, ,, | ,, ,, ,, |
| July | ,, ,, ,, | ,, ,, ,, |
| Aug. | ,, ,, ,, | ,, ,, ,, |
| Sept. | ,, ,, ,, | ,, ,, ,, |
| Oct. | ,, ,, ,, | 1st permanent incisors erupting in one sheep. |
| Nov. | 1st permanent incisors erupting in one sheep. | 1st pair erupting in two sheep. |
| Dec. | 1st pair erupting in two sheep. | 1st pair show roughened surfaces and badly splayed in one sheep. |
| 1948. | | |
| Jan. | 1st pair erupted in three sheep, and show roughened surfaces. | 1st pair erupted in three sheep; pitted and stained. |
| Feb. | All 1st incisors erupted, pitted and stained, but sturdier than in other groups. | All 1st incisors erupted, badly pitted and stained; teeth are sturdier than in other groups. |
| Mar. | No change. | No change. |
| Apr. | Some chipping of 1st pair. | 2nd pair erupting in one sheep; 1st pair show chalky areas, heavy erosion and slight chipping. |
| May | No change. | No change. |
| June | 1st pair heavily pitted and stained. | 1st pair heavily pitted and stained. |
| July | Chipping of 1st pair more pronounced. | 1st pair badly chipped. |
| Aug. | 1st pair badly chipped. | No change. |

*Group 7—continued.*

| Month. | Sub-Group 7A. | Sub-Group 7B. |
|---|---|---|
| Sept. | 2nd pair erupted in two sheep ; increased chipping of 1st pair. | 2nd pair erupted in two sheep ; increased chipping of 1st pair. |
| Oct. | No change. | No change. |
| Nov. | 2nd pair chalky, pitted and stained. | All 2nd incisors erupted, badly eroded and stained. |
| Dec. 1949. | Some chipping of 2nd pair. | 3rd pair erupting in one sheep. |
| Jan. | Striations, particularly on 2nd pair. | Marked striations on 2nd pair. |
| Feb. | 3rd pair erupting in one sheep. | No change. |
| Mar. | Damage intensified from 1st to 2nd to 3rd pairs of incisors. | Damage intensified from 1st to 2nd to 3rd pairs of incisors ; very bad chipping. |
| Apr. | All incisors eroded, heavily stained and badly chipped ; one deformed incisor arch. | Similar to 7A but damage more pronounced. |

After 12 months' continuous exposure to water containing fluorine, two animals were selected at random from each group and the first permanent incisors were photographed. The first permanent incisors of a normal sheep of the same age were also photographed for comparison. The abnormalities noted in the first pair of permanent incisors are referred to in the legends to Fig. 8-22.

Fig. 8.
Control.—The first pair of permanent incisors on a normal sheep.

Fig. 9.
Group 1A.—Erosion and some chipping of the cutting edges; the teeth badly overlap.

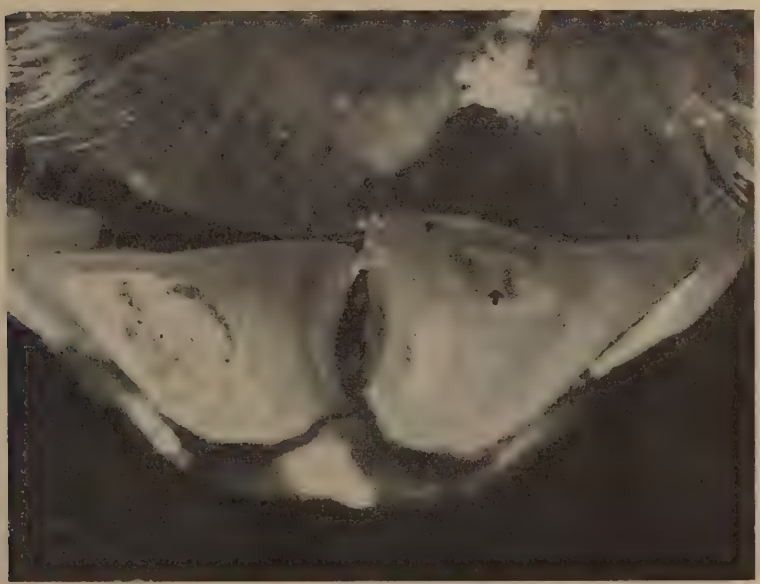


Fig. 10.
Group 1B.—Considerable erosion and two deep indentations on the left incisor (see arrow)

Fig. 11.
Group 2A.—Erosion.

Fig. 12.
Group 2B.—Considerable erosion and some chipping of the cutting edges.

Fig. 13.
Group 3A.—Considerable erosion and a deep indentation on left incisor (see arrow).

Fig. 14.
Group 3B.—Considerable erosion and transverse striations over lower two-thirds of the surface.

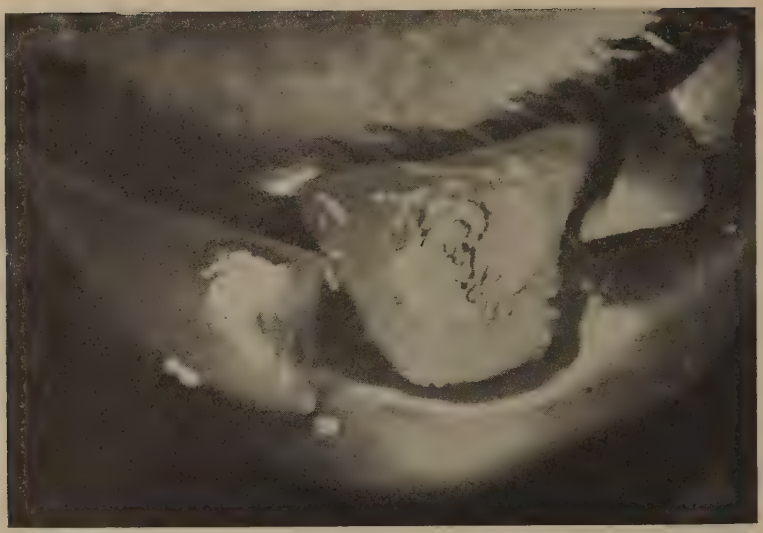


Fig. 15.

Group 4A.—Considerable erosion.

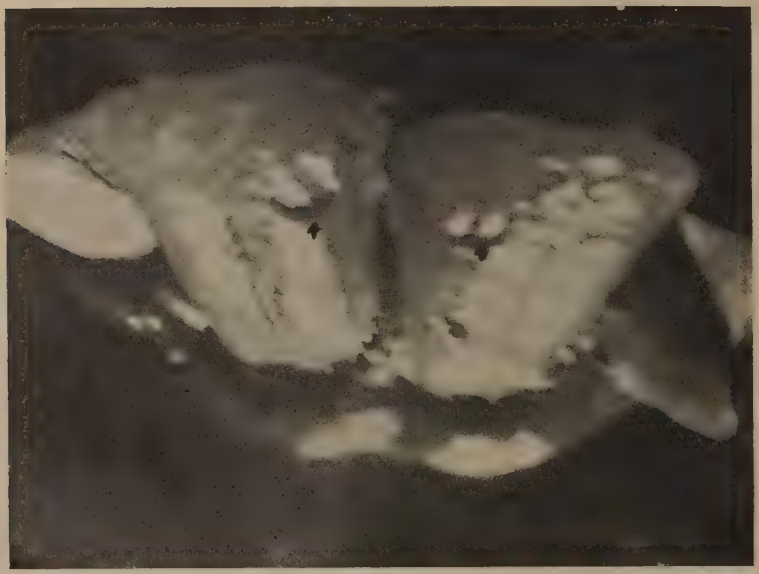


Fig. 16.

Group 4B.—Considerable erosion and some deep indentations (see arrows).

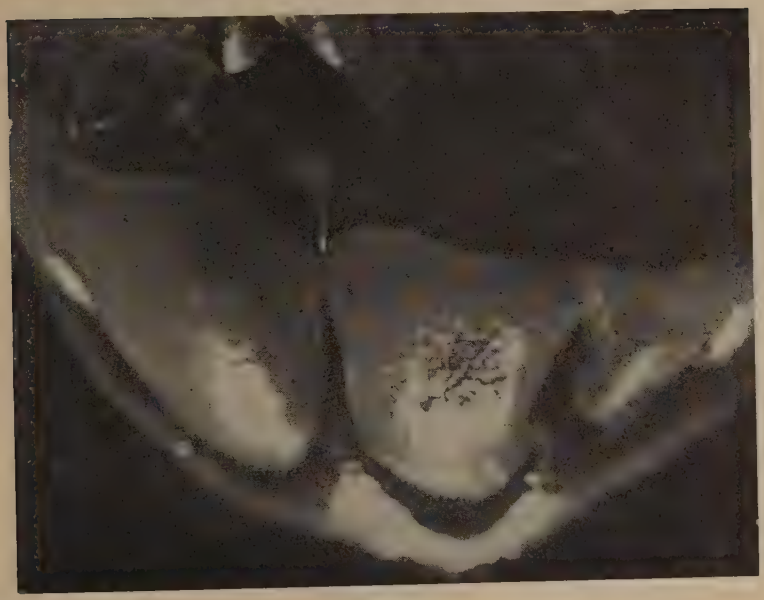


Fig. 17.
Group 5A.—Erosion.

Fig. 18.
Group 5B.—Considerable erosion and a deep indentation on right incisor (see arrow).

Fig. 19.

Group 6A.—Considerable erosion and some indentation (see arrows).

Fig. 20.

Group 6B.—Very marked erosion.

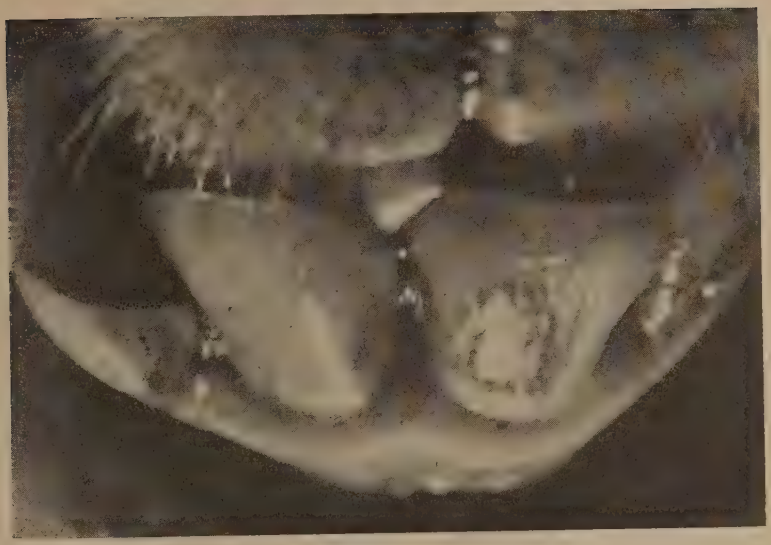


Fig. 21.

Group 7A.—Erosion.

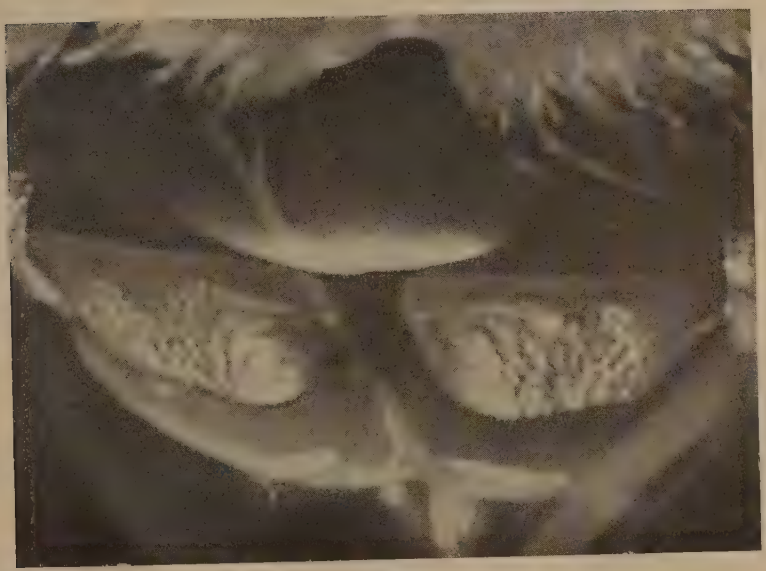


Fig. 22.

Group 7B.—Very marked erosion; much of the crown of the incisor is still embraced by the gum.

The experiment was carried on for two years. There were losses among the experimental animals, some from weakness, some from accidents, and one in group 1B apparently from hypocalcaemia.

At the conclusion of the experiment two animals from each group were slaughtered. At the same time two normal sheep of comparable age, but which had not been exposed to water containing fluorine, were selected as controls and the liver, kidney, pancreas, thyroid, heart (ventricle), gastrocnemius muscle, and duodenum prepared for analysis. These represent the edible portions of the animal and include the three kinds of muscular tissue—striated, smooth and cardiac.

Table 5 records the mean fluorine content of organs and tissues from animals in each of the groups.

**Table 5.**

FLUORINE CONTENT OF ORGANS AND TISSUE.

(p.p.m.F on dry matter.)

| Tissue or Organ. | Group. | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control. | 1A. | 1B. | 2A. | 2B. | 3A. | 3B. | 4A. | 4B. | 5A. | 5B. | 6A. | 6B. | 7A. | 7B. |
| Liver .. .. | 3·5 | 2·8 | 2·2 | 3·2 | 1·5 | 1·7 | 1·0 | 1·5 | 8·4 | 1·6 | 1·0 | 1·7 | 1·0 | 1·0 | 2·0 |
| Kidney .. .. | 4·2 | 14·8 | 22·6 | 8·8 | 14·6 | 10·4 | 32·8 | 9·2 | 16·8 | 10·4 | 14·2 | 26·2 | 43·4 | 6·2 | 5·4 |
| Pancreas .. .. | 2·8 | 1·0 | 5·2 | 2·8 | 5·0 | 1·7 | 1·5 | 3·0 | 2·0 | 1·4 | 1·6 | 2·8 | 5·0 | 1·0 | 2·0 |
| Thyroid .. .. | 3·0 | 4·6 | 13·0 | 7·0 | 7·3 | 8·0 | 4·0 | 3·0 | 6·0 | 7·2 | 3·0 | 10·0 | 10·0 | 7·5 | 10·0 |
| Heart .. .. | 3·0 | 2·4 | 3·0 | 2·4 | 2·4 | 2·0 | 1·0 | 1·0 | 6·0 | 2·8 | 1·0 | 1·5 | 1·1 | 2·0 | 1·5 |
| Gastrocnemius muscle .. .. | 2·0 | 1·2 | 5·5 | 2·0 | 1·0 | 1·0 | 1·6 | 3·0 | 2·0 | 1·0 | 2·0 | 3·0 | 1·6 | 2·4 | 2·0 |
| Duodenum .. | 3·4 | 2·0 | 2·5 | 2·6 | 2·0 | 4·8 | 2·2 | 3·2 | 6·0 | 2·4 | 3·5 | 3·0 | 2·2 | 1·2 | 4·0 |

The following conclusions may be drawn from the analytical data in Table 5:—

(1) There is some accumulation of fluorine in the kidney and thyroid.

(2) The edible portions of organs or tissues from sheep which have had access for two years to fluorine in the drinking water (up to 10 p.p.m.F) do not contain sufficient fluorine to be a danger to man.

Table 6 records the fluorine level in bones and teeth from animals in each of the groups.

**Table 6.**

FLUORINE CONTENT OF BONES AND TEETH.

(p.p.m.F. on moisture-fat free basis.)

| Bone or Tooth. | Group. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control. | 1A. | 1B. | 2A. | 2B. | 3A. | 3B. | 4A. | 4B. | 5A. | 5B. | 6A. | 6B. | 7A. | 7B. |
| Femur .. .. | 225 | 1375 | 2475 | 1300 | 2600 | 1275 | 2150 | 1600 | 1600 | 1150 | 1975 | 1030 | 1750 | 825 | 970 |
| Tibia .. .. | 200 | 1025 | 1500 | 850 | 1700 | 800 | 1625 | 1275 | 850 | 700 | 1325 | 550 | 1450 | 600 | 805 |
| Metatarsus .. | 150 | 750 | 1375 | 725 | 1600 | 725 | 1425 | 1100 | 825 | 775 | 1200 | 525 | 1125 | 550 | 675 |
| Mandible .. .. | 220 | 1880 | 3120 | 1960 | 2000 | 1240 | 2400 | 2480 | 1800 | 1600 | 2080 | 1200 | 2480 | 910 | 1360 |
| 1st Incisor .. | 84 | 600 | 1040 | 520 | 1100 | 530 | 880 | 860 | 600 | 520 | 740 | 480 | 1120 | 480 | 520 |
| 2nd Incisor .. | 86 | 1110 | 1880 | 840 | 1600 | 860 | 1380 | 1160 | 1170 | 1040 | 740 | 740 | 1220 | 570 | 660 |
| 3rd Incisor .. | 120 | 1070 | .. | 1520 | 1540 | 630 | .. | 1270 | 1540 | 1180 | 1600 | 810 | 1760 | 820 | 680 |
| 3rd Premolar .. | 110 | 1140 | 2220 | 960 | 1520 | 920 | 1840 | 1300 | 1240 | 1170 | 1440 | 690 | 1460 | 750 | 830 |
| 2nd Molar .. | 100 | 740 | 1240 | 780 | 960 | 620 | 920 | 920 | 920 | 660 | 1040 | 400 | 1160 | 480 | 620 |
| 3rd Molar .. | 120 | 1210 | 2200 | 1120 | 1580 | 880 | 1400 | 1210 | 1230 | 1140 | 1560 | 760 | 1130 | 580 | 760 |
| Dentine .. .. | 210 | 1720 | 3000 | 1490 | 1970 | 1630 | 2000 | 2010 | 1440 | 1700 | 1640 | 1190 | 1680 | 720 | 1200 |
| Enamel .. .. | 60 | 660 | 930 | 610 | 510 | 480 | 1170 | 900 | 670 | 970 | 710 | 360 | 810 | 300 | 480 |

The combination histogram and scatter diagram (Fig. 23) emphasises the variation in fluorine accumulation in the groups.

Table 7 records the mean weight of cleaned air-dried bones from the experimental animals.

**Table 7.**

MEAN WEIGHT IN GRAMS.

| Group. | Mandible. | Femur. | Tibia. | Metatarsus. |
|---|---|---|---|---|
| Control .. | 65 | 65 | 57 | 24 |
| 1A .. | 54 | 41 | 33 | 17 |
| 1B .. | 41 | 41 | 30 | 18 |
| 2A .. | 54 | 30 | 37 | 17 |
| 2B .. | 70 | 56 | 50 | 20 |
| 3A .. | 59 | 43 | 41 | 17 |
| 3B .. | 47 | 46 | 45 | 22 |
| 4A .. | 55 | 44 | 38 | 18 |
| 4B .. | 40 | 38 | 35 | 18 |
| 5A .. | 57 | 54 | 46 | 18 |
| 5B .. | 56 | 60 | 55 | 20 |
| 6A .. | 53 | 47 | 42 | 18 |
| 6B .. | 59 | 63 | 55 | 25 |
| 7A .. | 62 | 50 | 41 | 20 |
| 7B .. | 55 | 51 | 45 | 20 |

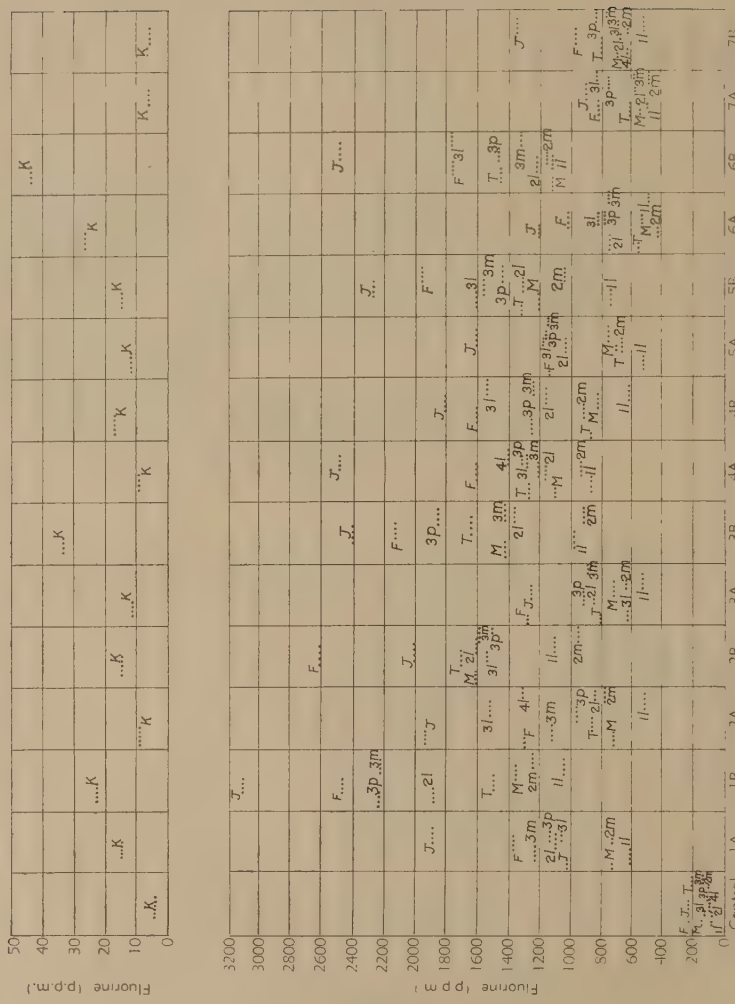



Fig. 23.

Diagram Showing Fluorine Accumulation in Kidney, Bones and Teeth of Sheep in each Experimental Group. K, kidney; F, femur; T, tibia; M. metatarsus; J. lower jaw; 1I, 1st incisor; 2I, 2nd incisor; 3I, 3rd incisor; 4I, 4th incisor; 3p, 3rd premolar; 2m, second molar; 3m, 3rd molar.

Some broad conclusions can be formed by examination of the analytical data in Tables 6 and 7.

(1) All groups show a considerable fluorine concentration in bones and teeth as compared with that found in the normal control sheep.

(2) In general, the fluorine content is greater for sub-groups B, where animals received water containing 10 p.p.m.F, than for sub-groups A, where animals received water containing 5 p.p.m.F. The concentration of fluorine in bones and teeth, however, is not proportional to fluorine intake.

(3) In the bones examined, the fluorine storage is greatest in the mandible, then in the femur, tibia and metatarsus, in that order.

(4) In the permanent incisor teeth, the fluorine storage in general increases from first pair to second pair to third pair.

(5) For the premolars and molars, the fluorine content varies with time of eruption, being lowest in the second molar and comparable in the third premolar and third molar.

(6) The fluorine concentration is much greater in dentine than in enamel.

(7) The greatest concentration of fluorine is in bones and teeth of animals from group 1B—the group on the basal ration and water containing 10 p.p.m.F.

(8) The lowest concentration of fluorine is found in the bones and teeth of animals from group 7—the lucerne group. This cannot be accounted for by the slight weight increase in bone noted in this group. It is in agreement, however, with metabolism studies on the accumulation and excretion of fluorine (see below), where it was found that the excretion was much higher in this group.

Photographs of the incisor teeth, taken at the end of the experiment from a single animal in each group, have been selected for illustration. It will be observed that staining of the incisors is noticeable only in group 7—the lucerne group. Comparison with "field" photographs shows that, under grazing conditions, staining defines the dental lesions of fluorosis more clearly. The abnormalities noted in the incisor teeth are mentioned opposite Figs. 24-38.

At the conclusion of the experiment, X-ray photographs of the femur, tibia and mandible of one animal from each group were taken. The mandibles were given 1 second exposure at 50 Kw, and the femur and tibia 2 seconds at 50 Kw. "Kodinex" was used.

Fig. 24.

Control.—Incisor teeth from a normal sheep of approximately the same age as the experimental animals.

Fig. 25.

Group 1A.—First pair show erosion and horizontal bands of pitting over the lower third; second pair show considerable erosion over the whole surface and bands of pitting; all teeth are paper white and dull, the first pair are elongated and badly splayed and there is some crowding of the second pair.

Fig. 26.

Group 1B.—First pair show erosion over most of the surface and some horizontal bands of pitting; second pair are chalky and very heavily pitted; all teeth are paper white and dull, the first pair are elongated and badly splayed and the second pair are at right angles to the normal plane.

Fig. 24.

Fig. 25.

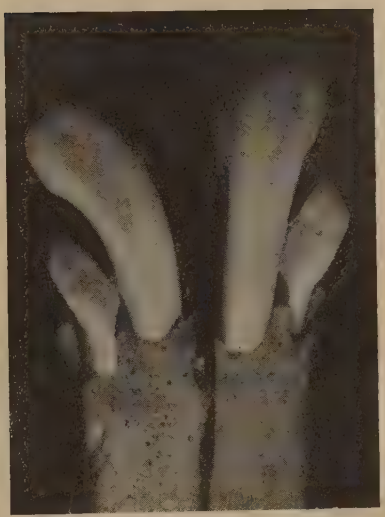


Fig. 26.

Fig. 27.

Group 2A.—Both the first and second pair are paper white and show some erosion; the first pair are elongated; the second pair are crowded and show roughening of the cutting edges.

Fig. 28.

Group 2B.—First pair show erosion over the lower two-thirds and bands of pitting at the base; the second pair are chalky, very heavily pitted and there is some roughening of the cutting edges.

Fig. 29.

Group 3A.—Both first and second pair are paper white and show erosion, more pronounced in the second pair; the first pair show chipped cutting edges and some chalky areas; the second pair are badly crowded.

Fig. 30.

Group 3B.—A badly deformed incisor arch; the breaking of the right first and second incisor occurred after death, but indicates the extreme brittleness of the teeth; the first pair are eroded with deep bands of pitting towards the base, the cutting edges are badly chipped and there are both vertical and horizontal fractures; the second pair are more heavily eroded and show horizontal striations of chalkiness.

Fig. 27.

Fig. 28.

Fig. 29.

Fig. 30.

Fig. 31.

Group 4A.—The first pair are eroded, show some deep indentations and there are bands of pitting towards the base; the second pair are very heavily eroded and show bands of pitting; all incisors are paper white and very chalky.

Fig. 32.

Group 4B.—A badly deformed incisor arch; the first pair are splayed, one of the second pair has erupted at right angles and the third pair are badly splayed; all incisors are paper white and chalky; the first pair are eroded, there are some deep indentations, there are bands of pitting at the base and the cutting edges are slightly chipped; the second pair are markedly eroded and pitted in bands; the third pair are particularly chalky, very heavily eroded and deeply pitted in bands.

Fig. 33.

Group 5A.—The first pair show some erosion and a tendency to banding at the base; the second pair are duller and more eroded; the third pair are slightly at right angles to the noromal plane, are chalky and show pronounced erosion.

Fig. 34.

Group 5B.—The first pair show considerable erosion, there are bands of pitting (one band being particularly pronounced), and there is slight chipping of the cutting edge; the second pair are markedly eroded with several pronounced bands of pitting towards the base; all incisors are paper white and extremely chalky.

Fig. 31.

Fig. 32.

Fig. 33.

Fig. 34.

Fig. 35.

Group 6A.—The first pair show erosion and a tendency to bands of pitting towards the base, there is some chipping of the contact surfaces and vertical cracks extending from the cutting edges; the second pair are more heavily eroded.

Fig. 36.

Group 6B.—The first pair show marked erosion with definite bands of pitting towards the base; the second pair show extremely heavy erosion with some very deep horizontal bands of pitting; there is a pronounced exostosis (see arrow) on the base of one of the second incisors; all incisors are very chalky and there is some staining.

Fig. 37.

Group 7A.—The first pair show erosion with two defined horizontal bands of pitting; the second pair show very marked erosion and horizontal bands of deep pitting; the third pair, though not clearly defined in the photograph, closely resemble the second pair; all incisors are very chalky and there is some staining.

Fig. 38.

Group 7B.—In this sheep much of the crown of the incisors is still embraced by the gum; all incisors are heavily stained above gum level, paper white and lacking in lustre below gum level; all are heavily eroded and this is emphasised by staining and there is considerable chipping of the cutting edges.

Fig. 35.

Fig. 36.

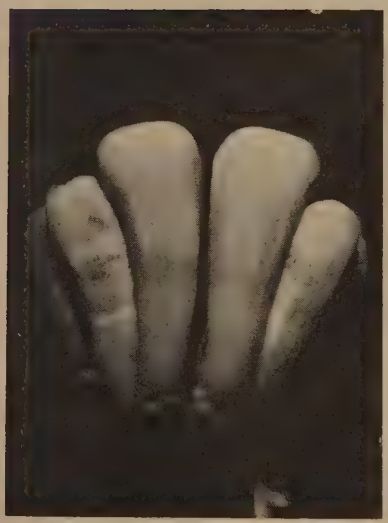


Fig. 37.

Fig. 38.

These X-ray photographs are presented to indicate the bone changes noted in the experimental animals at the conclusion of two years' exposure to water containing fluorine. The "normal" animal, although approximately the same age, had been kept under grazing conditions and the possible effects of exercise on bone development cannot be excluded. These photographs are not intended to illustrate the mode of action on bone structure of fluorine at low concentration in the drinking water ingested by the ruminant. Some of the abnormalities, particularly in those groups on a low plane of nutrition, are perhaps not entirely due to fluorine. However, these conditions of exposure to fluorine do occur in Queensland, at least in some seasons, and bone and dental abnormalities are enhanced.

The following comments are made on the photographs, which appear as Figs. 39-68.

Fig. 39.

Control.—Femur and tibia of a normal sheep of approximately the same age as the experimental animals. This animal, unlike those in the experimental group, was not penned; it had access to grazing plus lucerne chaff.

Fig. 40.

Control.—Side view of a normal mandible.

Fig. 41.

Group 1A.—Femur and tibia are smaller than the normal, the compact substance is much thinner, and the ossification of the epiphyseal cartilages is not as advanced as in Fig. 39.

Fig. 42.

Group 1A.—The mandible is shortened; the angle of the mandible commences at the anterior end of the root of the third molar; a crowding of the molars has resulted and this must at least partly account for the irregular cutting surfaces of the molar and premolar teeth compared with the normal; there is very little compact bone under the roots of the second and third molars; the roots of the second and third molars have produced irregularities on the ventral border of the mandible, which prior to X-rays were thought to be exostoses.

Fig. 43.

Group 1B.—The femur and tibia show similar effects to those in Fig. 41; the compact substance is again very thin and the epiphyseal cartilages are prominent.

Fig. 44.

Group 1B.—Very similar to Fig. 42; the horizontal ramus is short; there appears to be insufficient room for the complete eruption of the third molar; the first molar has overlapped the third permanent premolar; the compact substance is thin and the roots of the third molar have produced a well-defined irregularity on the ventral border of the mandible.

Fig. 45.

Group 2A.—The femur and tibia closely resemble those shown in Fig. 41.

Fig. 46.

Group 2A.—Very similar to Fig. 42; considerable shortening of the horizontal ramus; there appears to be insufficient room for the eruption of the third molar; roots of the second and third molars have produced a pronounced irregularity on the ventral border.

Fig. 47.

Group 2.—The femur and tibia are a little better calcified than the specimens from Group 2A, although the compact substance is still thin compared with the normal picture in Fig. 39.

Fig. 48.

Group 2B.—Some shortening of the mandible but not as great as in Figs. 44 and 46; the reduction in compact bone is not as great as in Groups 1A and 1B; there are no irregularities on the ventral border of the mandible.

Fig. 49.

Group 3A.—The femur and tibia resemble the specimen from Group 1A; the bones are smaller, the compact substance is no thinner but the medullary cavity is much reduced.

Fig. 50.

Group 3A.—This is comparable with the specimen from Group 1B; there has been very considerable shortening of the horizontal ramus and the angle of the mandible starts at the second molar; the compact substance is very thin and the roots of both the second and third molars have produced irregularities on the ventral border of the mandible.

Fig. 51.

Group 3B.—The femur and tibia are better calcified than specimens from Group 3A, but again the compact substance is thinner than in the normal specimen in Fig. 39.

Fig. 52.

Group 3B.—Some shortening of the horizontal ramus and there is a small irregularity on the ventral border of the mandible under the roots of the second molar.

Fig. 53.

Group 4A.—The femur and tibia show abnormalities similar to those in Fig. 41.

Fig. 54.

Group 4A.—The mandible shows considerable shortening, the angle of the mandible starting from the anterior end of the roots of the third molar; there would appear to be no room for complete eruption of the third molar; the compact substance is thin and all three roots of the third molar have produced irregularities on the ventral border of the mandible.

Fig. 55.

Group 4B.—The femur and tibia are comparable with the specimens from Group 4A.

Fig. 56.

Group 4B.—The mandible shows considerable shortening and resultant crowding of the molars; the roots of the third molar have produced small irregularities on the ventral border of the mandible.

Fig. 57.

Group 5A.—The femur and tibia are a little better calcified; the compact substance is more dense than in previous experimental groups but is still not comparable with the normal picture.

Fig. 58.

Group 5A.—There is some shortening of the mandible and the roots of both the second and third molars have produced irregularites on the ventral border of the mandible.

Fig. 59.

Group 5B.—The femur and tibia are comparable with those in Group 5A.

Fig. 60.

Group 5B.—Shortening of the mandible and the roots of the third molar extend into the thin shell of compact bone on the ventral border of the mandible.

Fig. 61.

Group 6A.—The femur and tibia are similar to those in Groups 5A and 5B.

Fig. 62.

Group 6A.—Considerable shortening of the mandible and crowding of the molars; the roots of the third premolar and all three molars extend into the compact substance on the ventral border of the mandible and have produced a number of small irregularities.

Fig. 63.

Group 6B.—The femur and tibia are better calcified and comparable with the normal picture in Fig. 39; there is an indication of a probable exostosis on the posterior surface of the distal third of the shaft of the femur.

Fig. 64.

Group 6B.—Some shortening but is more nearly comparable with the normal picture; however, unlike the normal, the roots of the second and third molar extend well into the compact substance.

Fig. 65.

Group 7A.—The femur and tibia are comparable with Group 6B and approach the normal picture in Fig. 39.

Fig. 66.

Group 7A.—This is very similar to Fig. 64.

Fig. 67.

Group 7B.—This plate closely resembles Fig. 65.

Fig. 68.

Group 7B.—This approaches more closely to the normal picture but there is still some shortening of the mandible and the roots of both second and third molars extend well into the compact substance.

Fig. 39.

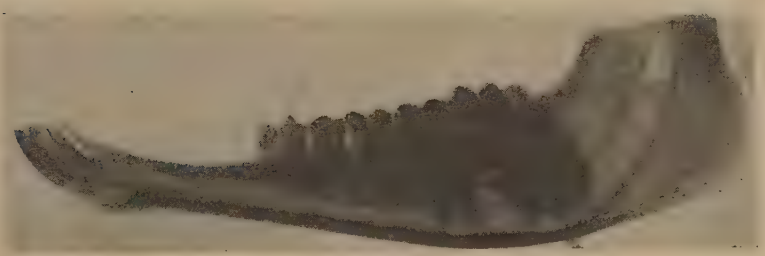


Fig. 40.

Fig. 41.

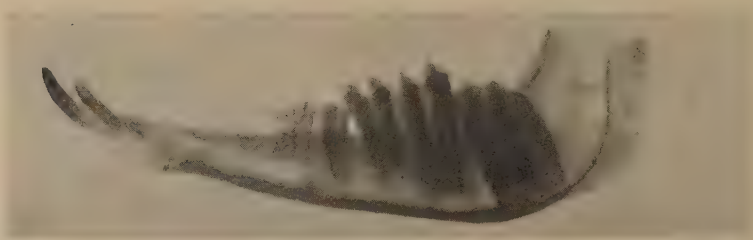


Fig. 42.

Fig. 43.

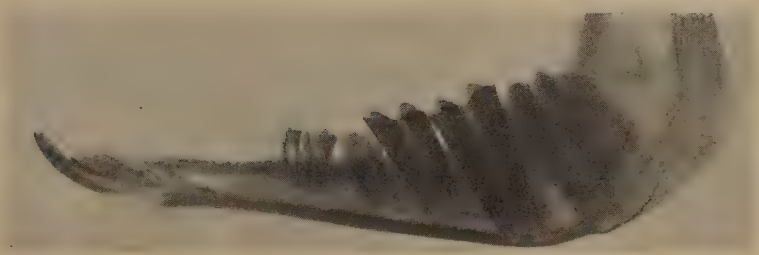


Fig. 44.

Fig. 45.

Fig. 46.

Fig. 47.

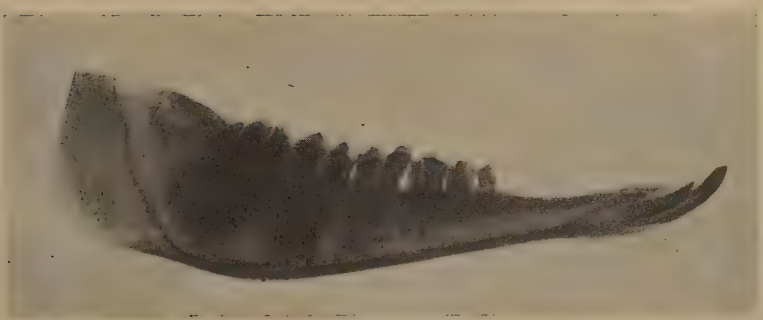


Fig. 48.

Fig. 49.

Fig. 50.

Fig. 51.

Fig. 52.

Fig. 53.

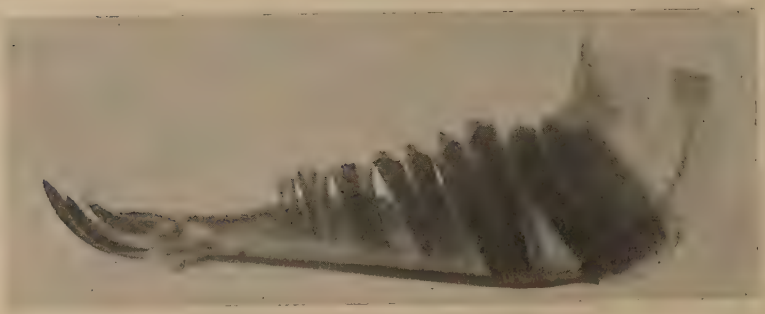


Fig. 54.

Fig. 55.

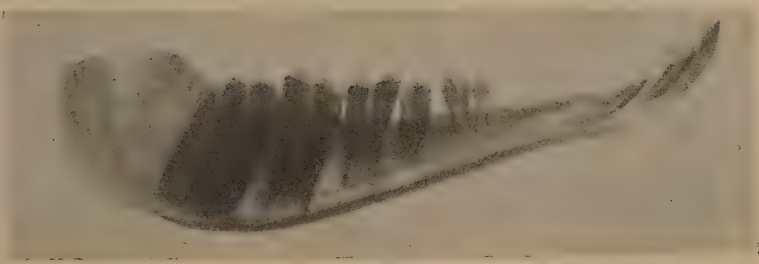


Fig. 56.

Fig. 57.

Fig. 58.

Fig. 59.

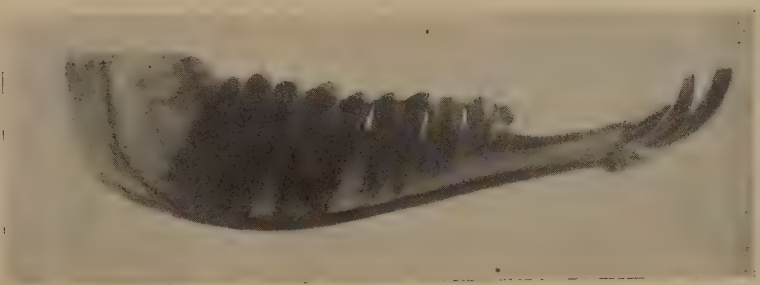


Fig. 60.

Fig. 61.

Fig. 62.

Fig. 63.

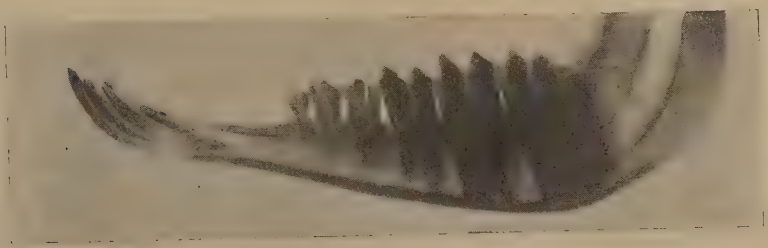


Fig. 64.

Fig. 65.

Fig. 66.

Fig. 67.

Fig. 68.

**Wool.**

Figs. 69-82 have been included to illustrate the condition of the wool grown by the experimental animals. The specimens were taken from the right shoulder at the conclusion of the investigation and represent about eight months' wool growth. A wool sample from one sheep in each group is shown.

The wool classer's description of the fleece from sheep in each experimental group was as follows:—

Group 1A.—Wool fine to medium, dull, short, poor character.

Group 1B.—Wool fine to medium, fairly dull, short, poor character.

Group 2A.—Wool medium, dull to fairly bright, fair length, poor character.

Group 2B.—Wool medium, fairly bright, fair length, poor character.

Group 3A.—Wool medium to strong, fairly bright, short to fair length, poor character.

Group 3B.—Wool medium to strong, fairly bright, fair length, poor to fair character.

Group 4A.—Wool fine to medium, dull, short, poor character.

Group 4B.—Wool fine to medium, dull, short, poor character.

Group 5A.—Wool medium, fairly bright, short to fair length, poor to fair character.

Group 5B.—Wool medium, fairly bright, fair length, poor to fair character.

Group 6A.—Wool medium, fairly bright, fair length, fair character.

Group 6B.—Wool fine to medium, fairly bright to bright, fair length, fair to good character.

Group 7A.—Wool medium to strong, bright, fair to good length, fair character.

Group 7B.—Wool medium, fairly bright to bright, fair to good length, fair character.

It was concluded that neither the quality nor the yield of wool was affected by fluorine intake though it was naturally obvious that the better wool came from the high level protein-fed beasts. Teeth damage did not prevent these experimental animals from handling the chaffed feed.

These experimental findings support field experience, which suggests that the teeth lesions plus the accompanying pain prevent the sheep from collecting and masticating the feed. Thus the nutritional level is reduced and in its turn the yield of wool. Further field evidence lies in the fact that actual wool defects were not recorded in sheep in Queensland prior to 1941, when White, Moule, and Seddon first looked for and found the dental lesions of fluorosis.

Fig. 69.
Group 1A.

Fig. 70.
Group 1B.

Fig. 71.
Group 2A.

Fig. 72.
Group 2B.

Fig. 73.
Group 3A.

Fig. 74.
Group 3B.

Fig. 75.
Group 4A.

Fig. 76.
Group 4B.

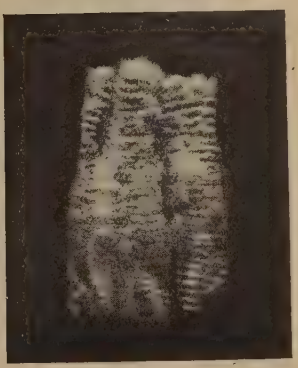


Fig. 77.
Group 5A.

Fig. 78.
Group 5B.

Fig. 79.
Group 6A.

Fig. 80.
Group 6B.

Fig. 81.
Group 7A.

Fig. 82.
Group 7B.

Fig. 83.
Upper and Lower Jaws of a Normal Animal.

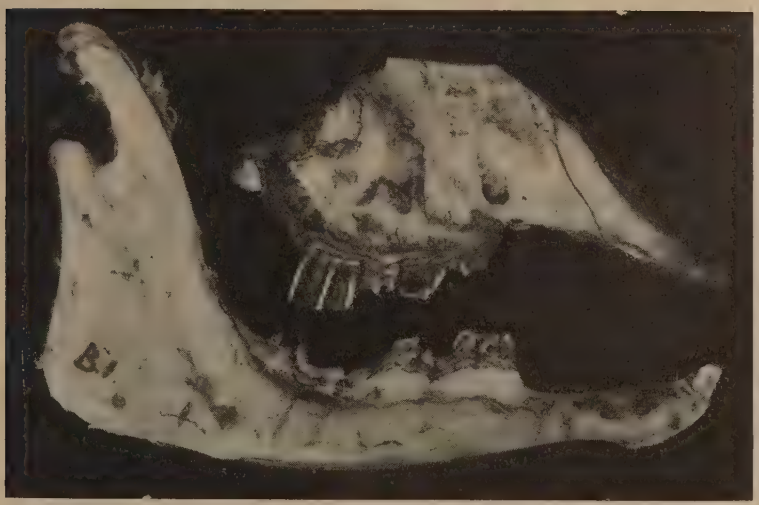


Fig. 84.

Abrasion of the Molars and Premolars of Sheep Using Water Containing 5–7 p.p.m. Fluorine and Depastured on Hard Mitchell Grass Country. Note the compensating wear on the upper and lower molars and premolars.

*Natural Cases.*—Figs. 83–86, from natural cases, show the damage done to incisor, premolar and molar teeth in sheep depastured on dry roughage and exposed to fluorided water. These illustrations were previously published by Moule (1945).

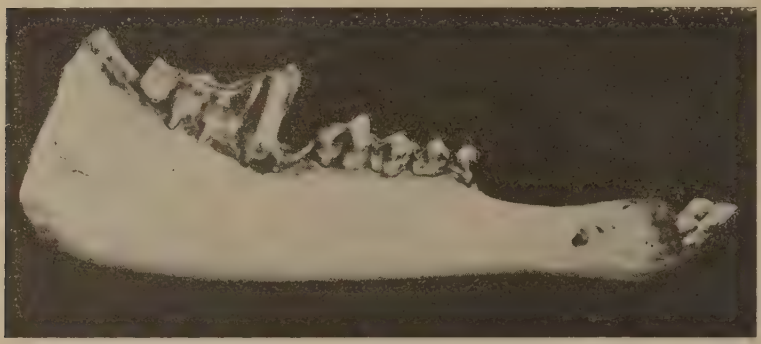


Fig. 85.

Uneven Wear on Molars and Premolars Under the Same Conditions of Exposure to Water Containing Fluorine.

It will be noted that in these field specimens the damage from fluorosis is emphasised by the accompanying staining. This is no doubt due to the presence of degradation products of chlorophyll from the pasture. Such products would be absent in the basal ration of oaten chaff fed to experimental sheep in the dietary mitigation studies.

Fig. 86.

Three Examples of the Effect of Fluorine Intake Through the Drinking Water on the Incisor Teeth of Sheep in Endemic Areas. Left, teeth from an animal following irregular intake of unsuitable water during its first year of life. Centre, teeth from a 6-tooth sheep on unsuitable water from an early age. Right, teeth from a sheep subjected to intermittent intake of unsuitable water during its growing period.

**Accumulation of Fluorine in Keratinous Tissue.**

No information on this aspect of the subject is available in the literature.

It was considered that it might be an index of the degree of exposure to fluorine of sheep in endemic areas. Samples of wool were taken from the right shoulder of selected sheep in each of the experimental groups, and hoof shavings were also collected. All specimens were thoroughly washed and dried before analysis. Table 8 records the fluorine content.

**Table 8.**

FLUORINE CONTENT OF WOOL AND HOOF.

(p.p.m.F on moisture-free basis.)

| Sample. | Group. | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1A. | 1B. | 2A. | 2B. | 3A. | 3B. | 4A. | 4B. | 5A. | 5B. | 6A. | 6B. | 7A. | 7B. |
| Wool .. | 4·8 | 12·4 | 4·6 | 10·8 | 7·6 | 7·6 | 9·2 | 5·6 | 6·0 | 6·8 | 11·6 | 5·6 | 5·6 | 4·4 |
| Hoof .. | 7·2 | 37·6 | 6·0 | 25·0 | 48·0 | 16·0 | 28·0 | 22·0 | 32·0 | 20·8 | 5·6 | 10·8 | 6·0 | 27·0 |

There appears to be no correlation between the intake of fluorine and its accumulation in keratinous tissue.

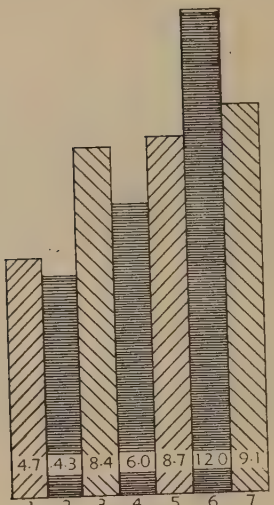
**Excretion of Fluorine.**

After 12 months' exposure to water containnig 10 p.p.m.F, the relationship between fluorine excretion in the urine or in the faeces and the concentration of calcium, phosphate and/or protein in the diet was examined.

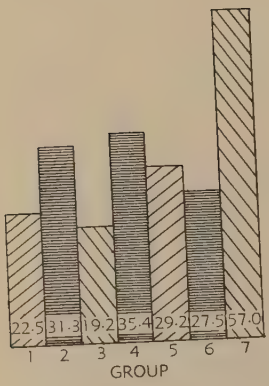
The procedure adopted was that outlined elsewhere by the author (Harvey, 1942). One wether from each group was placed in a metabolism cage. The feed intake was accurately weighed, the water consumption measured and the change in fluorine concentration corrected for the loss of water due to evaporation. The complete daily output of urine was collected by means of a tightly fitting rubber belt leading by a rubber tube to a collecting vessel. The faeces were collected by means of a canvas bag strapped to the animal. The food, water, urine and faeces were analysed for fluorine. All collections were made after an acclimatisation period of seven days. The histograms (Fig. 87) record the analytical data.

The fluorine intake varied widely for the different groups due to the variation in water consumption of individual animals. The daily water consumption, and hence fluorine intake, was much less than was desired, no doubt due to the animals being confined in boxes for the seven days prior to and the three days of collection, and the fact that the metabolism studies were conducted in winter.

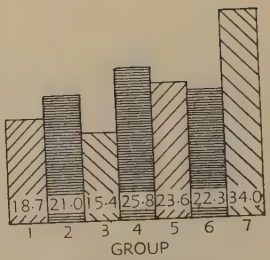
This experiment showed that there was no correlation between calcium, phosphate or protein intake and fluorine excretion. Fluorine excretion was greatest in the lucerne group—group 7. This is in agreement with analyses



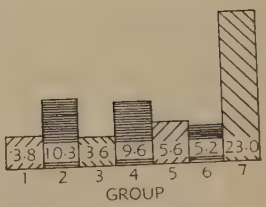

Daily Fluorine Intake in Milligrams.




Percentage Fluorine Excreted.




Percentage Fluorine Excreted in Urine.




Percentage Fluorine Excreted in Faeces.

Fig. 87.

Fluorine Intake and Excretion for One Experimental Sheep from each Group on Water Containing 10 p.p.m.F.

of bones and teeth from affected animals at the conclusion of the dietary mitigation studies, when it was found that fluorine storage was less in this group.

**Conclusions from Dietary Mitigation Studies.**

(1) On water containing either 5 p.p.m.F or 10 p.p.m.F, all animals in all experimental groups showed definite symptoms of fluorosis.

(2) Wear of the incisors was much less severe and abrasion of the molars was considerably less than in sheep in endemic areas.

The following explanations are offered to account for these differences:—

(*a*) Animals in the experimental groups were all on chaffed feed and had neither to bite nor to masticate the harsh fodder which is normally the natural source of feed for a considerable portion of the year in endemic areas.

(*b*) Because of lack of exercise, type of fodder and climatic differences, the water consumption (10 to 14 litres for each sheep weekly) was less than that of sheep in endemic areas.

(3) No significant beneficial effects were obtained from any of the ameliorative treatments used. The condition of the animal was better in those groups on a high protein ration, but all groups showed the definite lesions of fluorosis. It must therefore be concluded that—

(*a*) A treatment such as feeding a high protein, high lime or high phosphate ration, found to be beneficial in combating fluorosis in small laboratory animals, is not applicable to ruminants.

(*b*) Treatments recorded in the literature as beneficial to ruminants where fluorine has been fed in the ration are not applicable where the fluorine is administered in the drinking water.

(4) Fluorine administered in the drinking water has no deleterious effects on wool *per se*, though badly affected animals are unable to gather or masticate pasture and this is reflected in both the quantity and the quality of the wool produced.

(5) On water containing up to 10 p.p.m.F (this would correspond to very bad field conditions), the storage or accumulation of fluorine in the edible portions of the sheep, after two years' exposure to such water, is not high and would not constitute a danger to humans.

(6) Teeth—Apart from the usual lesions associated with fluorosis, there are indications that fluorine in the drinking water delays the eruption of the incisors and produces badly deformed mouths. The elongation of the incisors and some abnormalities may be partly due to the type of feeding and mineral imbalance. There were, however, cases of delayed eruption and deformed mouths in groups 6 and 7, where the animals were on a balanced diet.

(7) Bones—The findings shown in the X-rays are not in keeping with those reported by other workers. All experimental groups showed rarification rather than thickening of the bones. This is particularly noticeable in the mandible, where the irregularities on the ventral border, formerly thought to be exostoses, have been shown to be due to a reduction in thickness of compact substance and the roots of the molars (and in some cases the premolars) have extended well into the shell. Shortening of the horizontal ramus of the mandible is apparent in all groups. These features can be accounted for only partly by the type of feeding, by mineral imbalance, or by calcium and or phosphate deficiency. In any case mineral imbalance and phosphorus deficiency are not uncommon in most of the endemic areas of Queensland.

## ELIMINATION OF FLUORINE FROM ARTESIAN WATER.

Van der Merwe (1940) discussed methods of elimination of fluorine from water. Such treatments, however, are not economically possible on affected artesian water for stock in Queensland.

The use of a commercial deionising resin was examined. Water containing 10 p.p.m.F was run through a tower containing a mixture of acid and base exchange resins. The flourine level fell to 0.5 p.p.m.F. This process is effective and may be of use on a small scale for human consumption (e.g., in schools situated in endemic areas and where no alternative source of fluorine-free water is available). The cost of the resins, even without equipment for their use and regeneration, must make any such system of treatment impracticable for stock-watering purposes.

An attempt was made to use small quantities of lime and/or superphosphate as a fluorine precipitant. It was thought that, if these treatments proved benefical, it would be practicable to dust terminal dams or tanks with one or both of these precipitants. It was decided that on the score of cost the upper limit must not exceed 200 lb. of precipitant to one million gallons of water.

The following treatments were examined using this concentration:—

(1) Water containing 10 p.p.m.F plus calcium oxide at 20°C.
(2) Water containing 10 p.p.m.F plus calcium oxide at 40°C.
(3) Water containing 10 p.p.m.F plus calcium oxide after boiling and cooling.
(4) Water containing 10 p.p.m.F plus superphosphate at 20°C.
(5) Water containing 10 p.p.m.F plus superphosphate at 40°C.
(6) Water containing 10 p.p.m.F plus superphosphate after boiling and cooling.
(7) Water containing 10 p.p.m.F plus calcium oxide plus superphosphate at 20°C.
(8) Water containing 10 p.p.m.F plus calcium oxide plus superphosphate at 40°C.
(9) Water containing 10 p.p.m.F plus calcium oxide plus superphosphate after boiling and cooling.

The water was analysed immediately after treatment and at intervals of one, three and seven days. No reduction in fluorine level was achieved by any of these treatments.

It was mentioned previously that it was not possible to maintain water at 10 p.p.m.F in the 44-gallon drums to which calcium sulphate had been added. The presence of calcium sulphate was therefore put forward as an explanation of the reduction in fluorine level in waters reticulated by extensive bore drains through "kopi" country. Large deposits of calcium sulphate occur naturally in many parts of western Queensland. It could be added to drinking water either in troughs or terminal dams, and so offer a possibility of at least reducing a high level of fluorine. It was decided to investigate this possibility on a laboratory scale. Fig. 88 illustrates the findings.

It was found that in the laboratory the process is very slow. There appears to be some factor in the "synthetic" bore water prepared in the 44-gallon drum which accelerates this precipitation. For this reason sodium chloride, sodium bicarbonate and iron oxide were added and the temperature varied. There was still no appreciable increase in the rate of fluorine precipitation. This slow rate of fluorine precipitation with calcium sulphate must eliminate the use of this method as a practical control measure in endemic areas.

It seems that there is no practical method of economically eliminating or even reducing the fluorine content of artesian water for stock in endemic areas of Queensland. The methods of reducing evaporation suggested in the next section might be effective in reducing the concentration of fluorine in the water.

## MANAGEMENT OF FLOCKS IN ENDEMIC AREAS.

The results of the experiments on dietary mitigation and on the reduction of the fluorine level in the water having revealed no practical means of overcoming the problem of fluorosis in sheep, further information must be obtained on how flock management affects the severity of the lesions. From clinical observations, it has been shown that, by efficient management and the use of surface water for sheep during their susceptible period when permanent teeth are being laid down, the harmful effects of fluorosis can be largely avoided.

Surface water is conserved in many areas of the State not supplied by permanent streams. Water conservation is effected by making use of natural contours and providing earthen tanks for the collection and storage of run-off water drained in this manner. Sheep may water directly from the earthen tank, but it is quite common to pump water out to drinking troughs, as this prevents damage to the banks of the tank by the sheep. The conservation of surface water in western Queensland is limited by the porosity of the soil and the high evaporation from a free water surface.

Many soil types are unsuitable for holding water. This problem, however, may now be largely overcome by the use of dispersing agents to seal tanks and protect against the action of soft water.

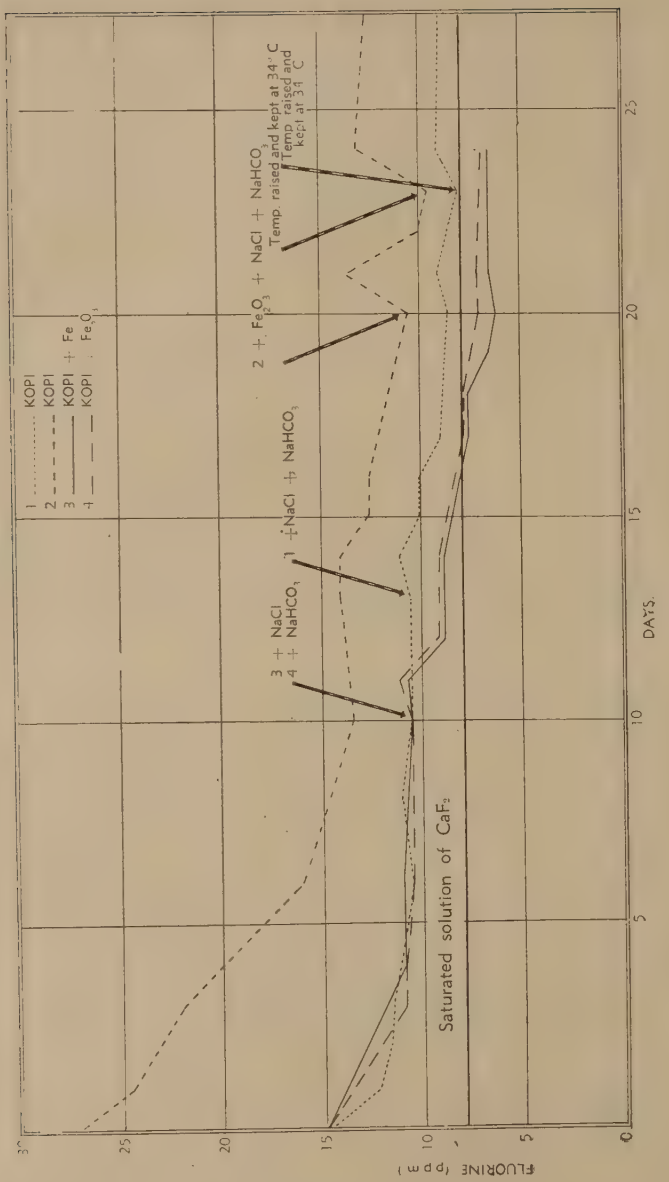



**Fig. 88.**

**Effect of Calcium Sulphate (Kopi) on the Fluorine Content of Water.**

As most of the rain falls during a short period of the year, a permanent water supply is difficult to assure, especially in drought years. Probably much could be done to reduce the high evaporation loss from tanks by providing wind shelters to minimize the evaporation due to wind, planting shade trees adjacent to tanks and dams, and floating on water surfaces raft-like coverings constructed of light wood and providing an air insulation space.

In spite of the difficulties presented by soil type and evaporation, surface water must be conserved in endemic areas, particularly on properties where all available artesian water contains a harmful level of fluorine. This is essential even if the water made available by this means is only sufficient to meet the requirements of young stock and for limited periods.

Consideration must be given to the alteration of paddocks by movement of fences and to inter-paddock movements of sheep. At no time should areas be too large or water facilities so arranged that sheep have to walk long distances to water. The following considerations must enter into the management of flocks in fluorided areas:—

(1) Young susceptible sheep should be maintained in paddocks watered by surface water or fluorine-free artesian water.

(2) Grown sheep may be held in paddocks watered by affected artesian water (up to 10 p.p.m.F).

(3) Breeding stock may be held on affected water but should be moved to surface water when the lambs start to drink.

(4) Sheep of various ages should be so rotated between paddocks containing affected and non-affected water that the most effective use is made of all grazing facilities and minimum damage is done to sheep through fluorosis.

Further studies on this aspect of fluorosis are now in progress with the object of determining how the effects of fluorosis can be mitigated by variation in the time of exposure to and protection from water containing fluorine.

## GENERAL CONCLUSIONS.

1. Supplementary feeding of protein, lime and/or phosphate does not mitigate or delay the onset of fluorosis in sheep using water containing either 5 or 10 p.p.m.F as their sole drinking supply.

2. Up to 10 p.p.m.F in the drinking water affects wool production in sheep only in so far as the dental damage limits food consumption.

3. There is no appreciable accumulation of fluorine in any edible portion of sheep which have been for two years on water containing 10 p.p.m.F. Hence, there is no danger to humans in the consumption of such animals.

4. Pregnant ewes drinking water containing up to 10 p.p.m.F do not transmit appreciable quantities of fluorine either to the foetus or to the lamb through the milk after birth. However, although this procedure is safe with regard to the lamb, field evidence does suggest lower lambing figures in such ewes. In addition, water containing 10 p.p.m.F is certainly harmful to young breeding ewes.

5. Information has been collected to explain anomalies in the incidence of fluorosis in endemic areas.

(*a*) Pasture growing along bore drains or on land flooded by bore drains reticulating fluorided water may take up appreciable quantities of fluorine. This offers an additional source of fluorine to stock in affected areas.

(*b*) Soil forming the banks of bore drains and soil of areas flooded by bore drains reticulating fluorided water contain appreciable quantities of fluorine. In localities where earth eating by stock is common, this may offer an additional source of fluorine.

(*c*) Evaporation in bore drains, terminal dams, earthen tanks and troughs may raise sharply the fluorine level of the water. In general, these levels increase with distance from the borehead. A case was noted where the fluorine level decreased with distance from the borehead, and it was found that the bore drain ran through large calcium sulphate deposits.

(*d*) Clinical observations showed minimal fluorine damage to sheep in good or 'flush' seasons. The explanation is offered that under such circumstances most of the animals' water requirements will be met by moisture in the succulent green pasture together with temporary catchment of surface water.

(*e*) Alternative sources of water are often available for a portion of the year either from surface water or from one or more bores containing innocuous levels of fluorine. Inter-paddock movements must then be taken into account in the correlation of the incidence of fluorosis with the fluorine content of the water.

6. It is not possible economically to reduce the fluorine levels in affected artesian water to an innocuous level for stock.

7. In the light of present knowledge the only effective means of combating this disorder lies in the management of flocks. Investigations are now in progress to find the period of exposure to affected water which will produce the least damage in stock. Until these studies are complete, the following rules for flock management should be observed:—

(*a*) Surface water should be provided in some paddocks.

(*b*) Subdivision of paddocks should be based on the distribution of bore drains. Young stock should be pastured on surface water if possible; if not, on the bore drain containing the least fluorine (i.e., as near the borehead as possible).

(*c*) The use of paddocks watered by far distant portions of the bore drain or terminal dam should be restricted to mature dry stock. In a 'flush' season such paddocks may be used by young growing stock, but preferably for short periods and certainly not when the pasture reaches standing hay stage.

(*d*) If necessary, lambing ewes can be held on water containing a high fluorine level. Once the lambs are old enough to drink water—say three weeks—the ewes and lambs must be moved to surface water or water of mineral fluorine content, or the lambs must be weaned early and moved to safe water.

8. In the present state of our knowledge, extreme caution should be exercised in drilling new bores in an endemic area, although bores of low fluorine content have been found in areas where neighbouring bores show a high level of fluorine.

## ACKNOWLEDGMENTS.

Assistance in the conduct of the studies reported was rendered by many Departmental officers, including Dr. M. White, Dr. J. Legg (provision of housing facilities), Mr. A. K. Sutherland (treatment and post-mortem and pathological examinations), Mr. G. R. Moule (clinical survey data), Mr. W. J. Sanderson (photographs) and Mr. W. W. Manley (photographs and drawings). Prof. W. V. Macfarlane, of the University of Queensland, took many X-ray photographs, and other photographs were taken by Prof. S. F. Lumb and Dr. J. A. Sagar, also of the University.

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* * * *

## APPENDIX.

### NOTES ON ARTESIAN WATERS.

An artesian basin is the whole of an area within which pressure water exists and from which artesian or sub-artesian water is obtained by boring, together with the area occupied by the ground water contained in the upper and marginal unconfined portions of the water-bearing beds. The extent of the Great Artesian Basin of Australia is shown in Fig. 89.

Where the storage system is open on its upper side and the contained water supports only the pressure of the atmosphere, this water is known as ground water.

Where the cavities in which water is stored in the rocks are not interconnected with free access to the air, this water supports the pressure of the overlying column of water in addition to the pressure of the atmosphere and is known as pressure water.

The general term to include every kind of water-carrying bed, opening or cavity in rocks is aquifer.

When a borehole taps an aquifer containing pressure water, this water may be under such pressure that it rises above the surface of the ground and is said to be artesian water. The borehole from which it flows is termed an artesian bore (Fig. 90).

In those cases in which the pressure suffices to cause the water to rise above the level at which it is met, but does not suffice to cause it to rise above the surface, the water is said to be sub-artesian, and the bore is termed a sub-artesian bore.

The reticulation of artesian water is effected by cheaply constructed drains or channels of uniform depth with a minimum fall of nine inches to the mile (Fig 91). The fall is generally greatest from the spillway at the borehead where the borehole is situated. The length of the drain varies in different localities, but a flow of approximately 10,000 gallons per day is required for one mile of bore drain. The bore drain may terminate in a dam or discharge into a natural water channel or depression. Stock may water directly from the bore drain or the water may be pumped to drinking troughs.

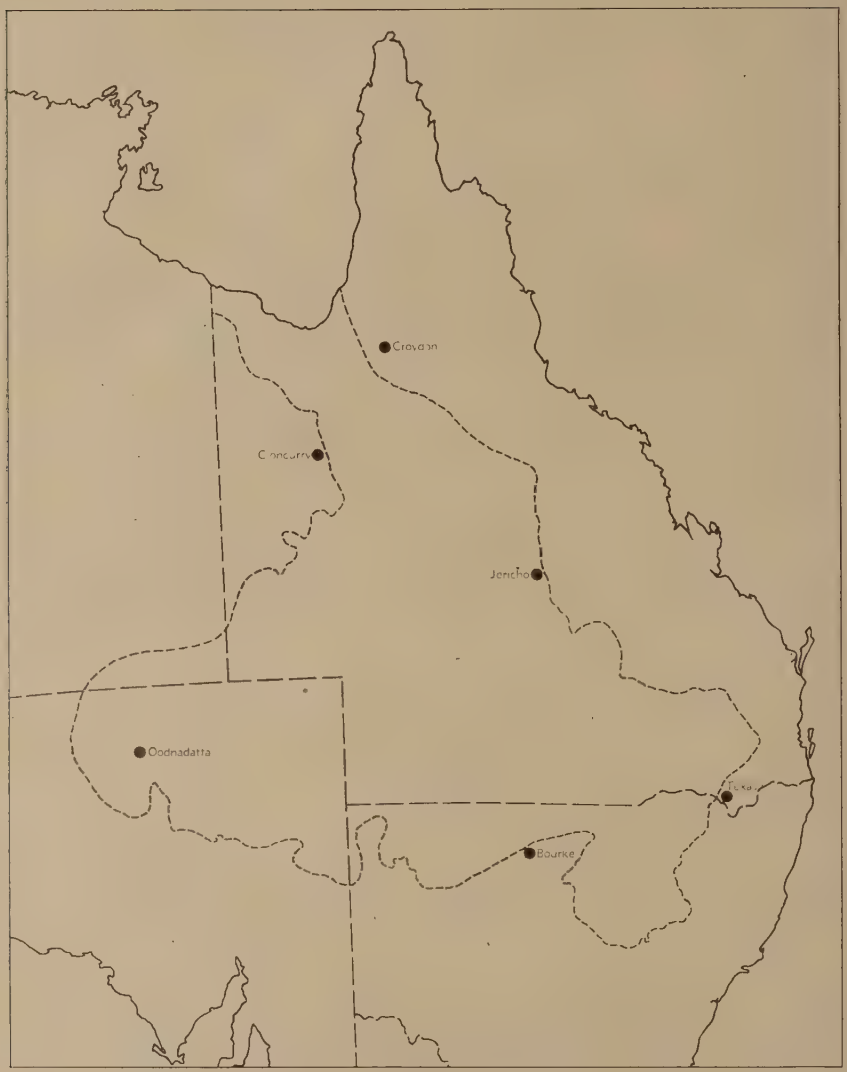


Fig. 89.

Map Showing Approximate Limits of the Great Artesian Basin.

[*From a map published by the Queensland Department of Public Lands.*

Fig. 90.
A Typical Borehead.

Fig. 91.
Portion of a Bore Drain.

# POTATO TUBER MOTH (*GNORIMOSCHEMA OPERCULELLA* (ZELL.)) INVESTIGATIONS IN SOUTHERN QUEENSLAND.

By A. W. S. MAY, M.Sc.Agr., Entomologist, Science Branch, Division of Plant Industry.


## SUMMARY.

*1. Three (sometimes two) applications of 1 lb. DDT per acre at fortnightly intervals prevented infestation of tops during most of the growing period of the spring-planted crop and reduced the likelihood of tuber infestation.*

*2. DDT was more effective as a spray than as a dust; sprays produced increases of up to 17% in number and 43% in weight of table quality tubers.*

*3. Hilling reduced tuber infestation and did not retard tuber formation when applied between 12 and 14 weeks after planting. Hilling alone did not prevent tuber damage, but following DDT spraying it served as an effective barrier against tuber infestation in the preharvest period.*

*4. The proper integration of DDT spraying and hilling is essential for Gnorimoschema control, as each fulfils a separate but complementary role in ensuring the maximum production of sound tubers.*


## INTRODUCTION.

The potato tuber moth (*Gnorimoschema operculella* (Zell.)) has long been recognized as one of the major pests of potatoes, being well established in many countries throughout the world. Larval tunnelling in the leaves (Fig. 1) and growing points reduces effective leaf area (Fig. 2), while tunnelling within the tubers reduces market value. It is by far the most important pest of potatoes in Queensland, occurring in all centres of production. In the southern part of the State, damage to tops sometimes occurs in the autumn-planted crop, but the pest rarely assumes major importance. In the spring-planted crop, however, high populations may develop, particularly during seasons of low rainfall, when total crop failures due to tuber moth attack are not uncommon.

Early attempts to control this pest with chemicals were handicapped by lack of effective insecticides and machinery for their application and by the excessive costs involved. Spray schedules incorporating arsenate of lead (Newman and Morgan, 1937) and other arsenical preparations (Lloyd, 1943 ; Helson, 1944) were unsuccessful. More promising results were obtained with derris (Lloyd, 1943) and phenothiazine (Helson, 1944) under experimental conditions, but costs of application limited their adoption on a field scale. For this reason emphasis was placed on cultural practices that would prevent or minimize damage in the field. Langford (1933) and Lloyd (1943) had found that deep planting combined with hilling as the crop developed were effective in reducing tuber infestation.

Measures applied prior to harvest gave indifferent results and additional practices were adopted to prevent further losses. Newman and Morgan (1937), Lloyd (1944) and others advocated rapid harvesting and the removal of the bagged tubers from the field as soon as practicable to reduce the likelihood of egglaying or infestation by migrant larvae once the tubers were exposed. More recently, the application of various types of insecticidal dusts to the bagged tubers has been shown to assist greatly in preventing infestation at this stage (Lloyd, 1944 ; Smith, 1944 ; Cannon, 1947 ; Helson, 1949).

With the advent of DDT, the outlook for tuber moth control in the tops changed considerably. Granovsky (1944), in preliminary trials, showed the worth of this insecticide against potato pests, while Cannon and Caldwell (1946) were able to prevent leaf mining in tobacco by *Gnorimoschema* larvae following DDT applications. Lloyd (1946), Cannon (1948), Helson (1949), and Hofmaster (1949) investigated the use of DDT to prevent damage to potato tops ; all concluded that this insecticide was superior to all other materials previously used and advocated its use in the general control programme. Though a schedule of DDT applications was shown to prevent top damage, complete tuber protection in the field was not always obtained. Both Cannon and Hofmaster considered that a schedule of DDT applications to the tops, followed by a system of hilling or ridging plants as tubers developed, would be required for effective tuber protection.

No work had been undertaken to evolve an effective schedule of DDT treatments and cultural measures of control wholly suited to the conditions pertaining in southern Queensland. Here, the pest is active soon after plants of the spring crop are through the ground in early August, and subsequent crop development occurs during a period favourable for pest development. Very high populations may be present as the crop reaches maturity.

To minimize crop damage, an effective control programme is required. To this end, experiments were carried out on the spring crop for three consecutive years, commencing in 1948. Broadly, these investigations sought information on the part played by both chemical and cultural treatments in ensuring the production of sound tubers at harvest, and any effects such treatments may have on yield of tubers.

## METHODS.

All trials were planted in accordance with grower practices under irrigation conditions in the Lockyer Valley, sets being placed at a depth of 4–6 inches, in rows approximately 3 feet apart with 15-inch spacing in the rows. Cultural practices such as application of water and fertilizers, weed control and soil tillage were left to the discretion of the farmer.

Randomized layouts were used. Plot size varied slightly in different trials, according to the area of land available, the rows numbering four or five and row length being between one and two chains.

All hills were constructed with horse-drawn single-row scufflers, fitted with the appropriate attachments.

Sprays were applied by a knapsack sprayer when the plants were small, and later by a power spray fitted with a twin nozzle hand-operated spray rod operating at 200–250 lb. per square inch nozzle pressure. Dusts were applied in the early morning by manually operated rotary dusters.

It was intended to apply the insecticide at the rate of 1 lb. DDT per acre at each application. Spray dosages in the vicinity of this figure were achieved during the 1950 season's experiment, but in the two previous seasons excessive top growth necessitated applications of 100—150 gallons of 0·1% DDT spray per acre.

Dust applications ranged from 30 lb. to 45 lb. of 2% DDT dust per acre, depending on the leafiness of the plants and the atmospheric conditions prevailing. Under ideal dusting conditions, adequate plant coverage was obtained with approximately 30 lb. dust per acre. A 2% dust was used because at a lower concentration a dosage rate approaching 1 lb. DDT per acre would be wasteful of material.

### 1948 Experiment.

This was concerned with determining the most appropriate time for applying insecticides and the efficacy of DDT in both spray and dust forms. The trials comprised two series; in one the plots received a 2% DDT dust and in the other a 0·1% DDT spray. The series were identical in design, which was a 4 x 4 layout duplicated on two separate farms.

The treatments in both series were as follows:—

(1) DDT applied at the first sign of moth activity, with a second application 10 days later; (2) in addition to treatments in (1) above, a preharvest treatment three weeks before harvest; (3) a preharvest treatment only, three weeks before harvest; (4) no insecticides.

In both series, all plots were uniformly hilled shortly after flowering commenced and the hills maintained until harvesting. Frequent light spray irrigation was applied to reduce soil cracking as the tubers developed.

Harvesting of all plots was completed in the same week.

### 1949 and 1950 Experiments.

Though hilling had been practised in the 1948 season's experiment, no valid conclusions concerning its role in protecting the tubers from damage could be drawn. Subsequent experiments were concerned mainly with evaluating the relative contribution of insecticide and hilling towards freedom from tuber damage at harvest.

Abnormal rains in the spring and early summer of 1949 interfered with the trials, which were therefore repeated in the spring of 1950.

In both years, three identical trials were laid down on different farms. The layout used was six randomized blocks each covering the three cultural treatments, with insecticidal treatments superimposed and randomized within each block.

The treatments were as follows :—

(*a*) *Cultural treatments* :—(1) No hilling—inter-rows scuffled only; (2) rows hilled soon after flowering; (3) rows hilled soon after flowering and again three weeks later.

(*b*) *Insecticidal treatments* :—(A) 0·1% DDT spray applied at the rate of 1 lb. DDT per acre; (B) 2% DDT dust applied at the rate of 1 lb. DDT per acre; (C) no treatment.

The initial insecticidal treatment was applied when *Gnorimoschema* adults were first noticed in the crop. Subsequent applications were timed to prevent an infestation developing in treated plots and were made at intervals of approximately two weeks.

### Assessing Results.

Estimates of pest activity within plots were made in the 1948 and 1949 seasons by counting the number of larval mines in a random sample of plants in each plot. Systematic sampling was not attempted during these two seasons.

In the 1950 experiment, a count of the number of larval mines in the terminals of a random sample of 20 plants from the inner rows of each sub-plot was made. A terminal consisted of the first six leaves below the growing point that measured not less than four inches in length. In addition, the presence or absence of larval infestation in the growing point was taken into account. At each sampling, a total of 360 terminals was counted for each treatment. Counts were made prior to each insecticidal application, with an additional count approximately 10 days before harvesting.

As each plot or sub-plot in the 1949 and 1950 experiments was harvested, the weight of tops from a sample of plants was recorded and the tubers from these plants sorted into the following grades :—

| | |
|---|---|
| First grade : | tubers weighing three ounces or more. |
| Second grade : | tubers of table quality less than three ounces weight. |
| Chats : | all tubers too small for table use. |

For each of the above grades, the number and weight of tubers and the number of tubers infested by tuber moth larvae were recorded.

The methods of taking plant samples were as follows :—

1948 experiment : Twenty consecutive plants from each of two inner rows per plot were sampled, a total of 320 plants per treatment.

1949 experiment : Ten consecutive plants from each of two inner rows of each sub-plot were sampled, giving a total of 360 plants per treatment.

1950 experiment : Five consecutive plants comprised a unit sample. Three such samples were taken from sections of the inner rows in each sub-plot, making a total of 54 samples (or a total of 270 plants) per treatment.

### 1951 Observations.

Though formal plots were not used, several commercial crops were kept under close and regular observation during the 1951 season. On these areas, insecticidal and hilling treatments conformed to a pattern suggested by the results obtained in the previous experimental studies. The worth of these treatments was assessed by observing the extent of top damage and tuber infestation at harvest.

## RESULTS.

### 1948 Experiment—Sprayed Series.

*Block A.*—This trial, on portion of a commercial block of certified Factor potatoes, commenced with the application of a 0·1% DDT spray to treatments 1 and 2 on September 13. Moths were prevalent before this treatment was applied, and though plants were only six to eight inches high and possessed on the average from four to six leaves per plant, larval damage was evident. A pretreatment estimate of *Gnorimoschema* activity showed a mean of 1·4 larval leaf mines per plant.

The application of DDT to the surrounding commercial crop coincided with the initial experimental treatment and temporarily checked moth activity. Moths were again active by September 23, when the plants were approximately 12 inches high, with flower buds evident on most of them. A second DDT application was then given to plots receiving treatments 1 and 2. By this time a considerable difference between plots in rate of plant growth was evident. Plants which had received DDT applications made good top growth and were virtually devoid of insect damage. In contrast, unsprayed plants showed extensive larval mining ; populations of *Austroasca viridigrisea* Paoli and *Macrosiphum gei* Koch were also present and contributed towards a ragged appearance of the plants and obvious checking of their growth.

The preharvest DDT application for treatments 2 and 3 was applied to the respective plots on October 22. By this time, extensive leaf mining had occurred in plots receiving treatments 3 and 4 and some tops carried little green foliage. Those plants receiving treatments 1 and 2 showed little damage at this stage, the plants continuing to grow vigorously.

This trial was harvested on November 11.

*Block B.*—This duplicate experiment was located on a portion of an area of certified Factor potatoes on a farm some distance from the companion block A.

A pretreatment count on September 13 of larval mines from 100 plants within the experimental area gave a mean of 1·2 larval mines per plant. Subsequently, the incidence of *Gnorimoschema* developed to a greater degree than that recorded on block A. No insecticides were used in the adjacent farm area.

DDT applications in the separate treatments were made on dates corresponding with those in block A. Growth responses in those plots receiving treatments 1 and 2, and the effect of DDT on the general insect population, were again evident.

Following harvesting on November 10, the data from these two blocks were summarized (Table 1). Mean values for weight of tops and weight and number of tubers in each grade were calculated on the basis of 40 plants per plot; the figures for percentage infestation are weighted averages of the separate percentage figures. The prevention of insect damage on top growth in plots receiving treatments 1 and 2 was reflected in the increased weight and number of tubers produced. Highly significant increases of 43 per cent. in weight and 17 per cent. in number of table quality tubers were recorded.

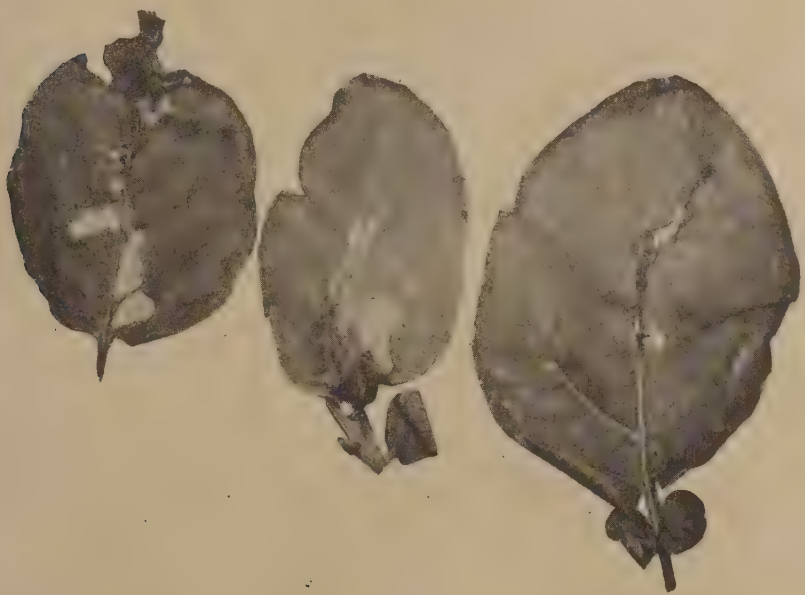

Fig. 1.

Potato Leaves Infested by *Gnorimoschema operculella* (Zell.). At this stage the larvae may still be active within the leaves.

**Table 1.**

SUMMARY OF DATA—1948 EXPERIMENT (SPRAYED SERIES).

| Treatment. | Mean weight of tubers. (oz.) | | | Mean number of tubers. | | | Mean weight of tops. (oz.) | Moth-infested tubers. % |
|---|---|---|---|---|---|---|---|---|
| | First and second grade. | Chats. | Total. | First and second grade. | Chats. | Total. | | |
| 1. Early spraying.. | 742 | 47 | 789 | 202 | 64 | 266 | 254 | 3·7 |
| 2. Early plus preharvest spraying .. .. | 713 | 52 | 765 | 186 | 68 | 254 | 302 | 2·4 |
| 3. Preharvest spraying .. .. | 493 | 39 | 532 | 161 | 57 | 218 | 193 | 7·3 |
| 4. Untreated .. | 501 | 42 | 553 | 156 | 71 | 227 | 107 | 11·2 |
| s.e. .. .. | 21·6 | 5·7 | 22·0 | 6·6 | 7·0 | 8·5 | 16·9 | .. |
| Significant differences— | | | | | | | | |
| 5% level .. | 64 | 17 | 65 | 20 | 21 | 25 | 50 | 2·0 |
| 1% level .. | 88 | 23 | 90 | 27 | 28 | 35 | 69 | 2·8 |

A preharvest treatment only, though prolonging top growth to a slight degree, had no influence on yield. DDT applications by reducing populations in the tops decreased the likelihood of tuber infestation; early treatments were more effective than the preharvest treatment in this respect.

## 1948 Experiment—Dusted Series.

*Block A.*—This trial, using the variety Sebago, was situated on the same farm and close to the sprayed series in block A. Thus somewhat similar levels of pest population could be expected for the two experimental blocks. A pretreatment count of larval activity in the tops within the dusted series gave a mean of 1·3 larval mines per plant.

Rain showers reduced the effectiveness of the dust deposit on the plants, falls being recorded within three days of both the first and second dust applications to treatments 1 and 2 on September 11 and 25. From the outset the dust did not appear to exhibit the same degree of control as the DDT spray and some moths were always present among the plants. An examination of 30 mines on leaves of both dusted and untreated plants on September 29, following the second application of dust, showed that 13 larvae were alive on the dusted plants and 24 on the untreated.

No obvious growth differences were noticeable between plants on dusted and untreated plots by September 29—a marked contrast to the obvious effect produced in the sprayed series nearby. However, all plants were responding to good growing conditions at this time, and it was obvious that the variety Sebago

was not affected by larval damage to the same extent as Factor. The Sebago plants were more upright and spreading, and the larger top growth tended to mask larval mining in the leaves.

The preharvest treatment was applied on the morning of September 23. All plots were harvested on November 12. The results, expressed as plot means, are given in Table 2.

**Table 2.**

DATA FOR 1948 EXPERIMENT (DUSTED SERIES).

| Treatment. | Mean weight of tubers. (oz.) | | | Mean number of tubers. | | | Mean weight of tops. oz. | Moth-infested tubers. % |
|---|---|---|---|---|---|---|---|---|
| | First and second grade. | Chats. | Total. | First and second grade. | Chats. | Total. | | |
| 1. Early dusting .. | 782 | 42 | 824 | 180 | 46 | 226 | 264 | 7·2 |
| 2. Early plus pre-harvest dusting.. | 687 | 50 | 737 | 153 | 49 | 202 | 294 | 6·5 |
| 3. Preharvest dusting .. .... | 476 | 39 | 515 | 133 | 43 | 176 | 168 | 6·8 |
| 4. Untreated .. | 551 | 30 | 581 | 137 | 34 | 171 | 155 | 7·6 |
| s.e. .. .. | 50·1 | 7·8 | 54·6 | 12·2 | 7·3 | 16·6 | 23·0 | .. |
| Significant differences— | | | | | | | | |
| 5% level .. | 160 | 25 | 175 | 39 | 23 | 53 | 74 | 3·2 |
| 1% level .. | 230 | 36 | 251 | 56 | .. | 76 | 106 | .. |

*Block B.*—This portion of the experiment, also laid down on the variety Sebago, was abandoned before harvest as unforeseen circumstances prevented uniform cultural management throughout the experimental area.

The results from block A revealed a similar trend as in the sprayed series with regard to weight and number of tubers and weight of tops produced for the several treatments. Early DDT applications resulted in increases of 42 per cent. in weight and 32 per cent. in number of tubers produced. However, variability between plots within treatments increased the necessary differences for significance. This can be attributed partly to uneven dust cover and the effect of rain in reducing the residual action of the dust.

No significant difference in percentage tuber infestation occurred between treatments. This could be explained partly by the reduced efficacy of the dust, but it is more likely due to a varietal characteristic. The varietal habit of producing long stolons increased the likelihood of the ground cracking, as the tubers tended to form nearer the soil surface; further, many tubers were partly exposed by hilling operations.

### 1949 Experiment.

Trials were located on three separate farms, and though some uniformity was possible in timing insecticide applications, unseasonable rainfall with resultant high soil moisture did not allow hilling to be undertaken at corresponding times on the three properties. Though interfering with the original plan of the experiment, this afforded an opportunity of evaluating the respective control measures under the abnormal conditions experienced.

Fig. 2.
Effective Leaf Area Reduced by the Larval Mining of *Gnorimoschema operculella* (Zell.).

### Trial 1.

In this trial, planted in mid-July to the variety Sebago, the majority of plants were flowering and had made large top growth by October 5. Excessive rain and its effect on the heavy clay loam soil prevented cultural treatment (2) being applied until treatment (3) was due. Both hilling treatments were carried out on November 1. The experiment was harvested on November 16.

The first DDT treatments, both spray and dust, were applied on October 5. Prior to this date *Gnorimoschema* was virtually absent, and only a very light infestation was evident when treatments were applied. A second insecticidal treatment was delayed by rain for so long that it was considered too late to be of any consequence for experimental purposes.

Though moths were always present in the plots after October 5, numbers were never high and the excessive growth of the tops masked pest activity. Adults of *Epilachna* 28-*punctata* Fabr. were prevalent after flowering, their damage being noticeable by harvest. The differences between treatments, particularly with regard to weight of tops produced, were largely due to this pest. Other pests, including *Austroasca viridigrisea*, were of little importance. The results, expressed as sub-plot means, are presented in Table 3.

**Table 3.**

DATA FOR 1949 EXPERIMENT (TRIAL 1).

| Insecticidal Treatments. | Cultural Treatments. 1. No Hilling. | 2. Early Hilling. | 3. Late Hilling. | Means. | Significant Differences. |
|---|---|---|---|---|---|
| | *(a) Mean weight of tubers from 10 plants (oz.)* | | | | |
| A (DDT spray) .. .. | 261 | 269 | 267 | 266 | |
| B (DDT dust) .. .. | 323 | 254 | 233 | 270 | |
| C (Untreated) .. .. | 276 | 254 | 250 | 260 | |
| Means .. .. .. | 287 | 259 | 250 | 265 | |
| | *(b) Mean number of tubers from 10 plants.* | | | | |
| A (DDT spray) .. | 75 | 68 | 62 | 68 | |
| B (DDT dust) .. .. | 76 | 70 | 66 | 70 | |
| C (Untreated) .. .. | 76 | 70 | 75 | 74 | |
| Means .. .. .. | 75 | 70 | 68 | 71 | |
| | *(c) Mean weight of tops of 20 plants (oz.)* | | | | |
| A (DDT spray) .. | 169 | 169 | 153 | 163 | A » B |
| B (DDT dust) .. .. | 134 | 159 | 121 | 138 | A > C |
| C (Untreated) .. .. | 145 | 140 | 144 | 143 | |
| Means .. .. .. | 149 | 156 | 139 | 148 | |
| | *(d) Percentage tuber infestation.* | | | | |
| A (DDT spray) .. | 6·4 | 2·2 | 3·6 | 4·1 | (1 > 2) |
| B (DDT dust) .. .. | 9·6 | 5·2 | 5·5 | 6·7 | B > A |
| C (Untreated) .. .. | 7·0 | 4·6 | 3·9 | 5·2 | |
| Means .. .. .. | 7·7 | 4·0 | 4·3 | 5·3 | |

In the final column, brackets indicate that the difference exceeds the necessary difference for significance but the F value in the analysis of variance is not significant.

> = significantly greater than at the 5% level.

» = significantly greater than at the 1% level.

Despite a low incidence of *Gnorimoschema* for the greater part of the experiment, hilling approximately two weeks before harvest was effective in slightly reducing percentage tuber infestation. This benefit was obtained despite the soil being too wet to permit the construction of proper hills. There is a suggestion that hilling was associated with a slight reduction in both weight and number of tubers produced, but the differences were not significant.

Insecticidal treatments were not associated with any increases in weight or number of tubers produced and were applied too early to have any influence on tuber infestation at harvest. Sprayed plots produced more top growth than dusted or untreated plots, an effect due largely to the incidence of *Epilachna* 28-*punctata* and allied foliage pests in the respective plots throughout October. Such damage was of little consequence, for it had no influence on the yields obtained.

**Trial 2.**

This area of the variety Sarenac on a heavy clay loam soil was planted in late August following late winter rains. *Gnorimoschema* was active by the beginning of October, and the first DDT treatment was applied on October 5. By this time, some plants were flowering but had not produced large tops. The second DDT treatment was delayed by rain until November 3 ; larval activity in the tops had been increasing for some time. The excessive top growth prevented good coverage being obtained. Further rains in mid-November prevented the application of a third insecticidal treatment ; there was evidence of *Gnorimoschema* activity at this time.

Recurrent rain and the constant wetness of the soil caused by excessive top growth interfered to some extent with the application of cultural treatments. The early hilling treatment was applied on October 17 when the majority of plants were flowering, but the wet, firm soil prevented the implements penetrating to a sufficient depth and these hills were poorly constructed. The late hilling treatment was applied on November 9, and again excessive soil moisture and intergrown plants prevented the formation of satisfactory hills. Maintenance of these hills was prevented by further rain.

Apart from *Gnorimoschema*, pests were of little importance. Any activity by *Epilachna* 28-*punctata*, *Austroasca* spp. and other leaf-feeding insects was masked by the copious leaf growth.

The variety Sarenac formed its tubers on unusually long stolons, some measuring over nine inches in length. Some tubers formed within the soil zone traversed by cultivating and hilling implements. No doubt hilling interfered with the setting or development of such tubers, while many were left partly exposed or only slightly covered with soil after the passage of the hilling implement. Soil packing from the heavy rains and the excessive growth of tops promoted mechanical damage to tops during hilling. This, in turn, would influence plant growth to a certain degree.

**Table 4.**

DATA FOR 1949 EXPERIMENT (TRIAL 2).

| Insecticidal Treatments. | Cultural Treatments. 1. No Hilling. | 2. Early Hilling. | 3. Late Hilling. | Means. | Significant Differences. |
|---|---|---|---|---|---|
| | *(a) Mean weight of tubers from 20 plants (oz.)* | | | | |
| A (DDT spray) .. | 516 | 436 | 473 | 475 | 1 » 2 ; 1 › 3 |
| B (DDT dust) .. .. | 484 | 390 | 382 | 419 | A » C ; A › B |
| C (Untreated) .. .. | 427 | 393 | 389 | 403 | |
| Means .. .. .. | 475 | 406 | 415 | 432 | |
| | *(b) Mean number of tubers from 20 plants.* | | | | |
| A (DDT spray) .. | 137 | 117 | 118 | 124 | 1 » 3 ; 1 › 2 |
| B (DDT dust) .. .. | 130 | 116 | 95 | 114 | A » C |
| C (Untreated) .. .. | 113 | 103 | 92 | 102 | |
| Means .. .. .. | 127 | 112 | 102 | 113 | |
| | *(c) Mean weight of tops of 20 plants (oz.)* | | | | |
| A (DDT spray) .. | 155 | 127 | 172 | 151 | |
| B (DDT dust) .. .. | 153 | 146 | 123 | 141 | A, B » C |
| C (Untreated) .. .. | 82 | 115 | 114 | 103 | |
| Means .. .. .. | 130 | 129 | 136 | 132 | |
| | *(d) Percentage tuber infestation.* | | | | |
| A (DDT spray) .. | 44·9 | 42·0 | 41·9 | 42·9 | |
| B (DDT dust) .. .. | 42·4 | 46·7 | 41·0 | 43·3 | (1 › 3) |
| C (Untreated) .. .. | 48·1 | 44·2 | 39·8 | 44·0 | |
| Means .. .. .. | 45·1 | 44·3 | 40·9 | 43·4 | |

Brackets indicate that the difference exceeds the necessary difference for significance but the F value in the analysis of variance is not significant.

› = significantly greater than at the 5% level.

» = significantly greater than at the 1% level.

Harvesting was unavoidably delayed for approximately two weeks until December 14, when extensive ground cracking was evident. *Gnorimoschema* moths were prevalent in the area and tuber infestation was severe when the experiment was eventually harvested. Table 4 sets out the results, expressed as sub-plot means.

The delay in harvesting nullified any benefits that DDT applications or hilling may have had on percentage tuber infestation. The waterlogged soil cracked extensively once the tops of the plants wilted and the surface of the soil became exposed to the sun. Under such conditions, the poorly-prepared hills

could not have been expected to fulfil their true function. *Gnorimoschema* populations from untreated plots and neighbouring areas of unsprayed potatoes found conditions ideal for the rapid reinfestation of DDT treatment plots.

Late hilling probably prevented tuber infestation to a slight degree, though the difference was not significant. Significant differences in favour of late hilling were obtained for percentage infestations in first grade tubers only (Table 5), suggesting that a greater proportion of these larger tubers than of the second grade and chats is formed deeper in the soil.

**Table 5.**

DATA FOR 1949 EXPERIMENT (CULTURAL TREATMENTS, TRIAL 2).

| | Cultural Treatments. | | | | Difference necessary for significance at 5% level. |
|---|---|---|---|---|---|
| | 1. No Hilling. | 2. Early Hilling. | 3. Late Hilling. | Means. | |
| First grade .. .. | 37·7 | 39·3 | 31·4 | 36·1 | 6·5 |
| Second grade .. .. | 51·7 | 47·5 | 47·8 | 49·0 | 7·8 |
| Chats .. .. .. | 52·8 | 49·5 | 52·7 | 51·7 | 10·0 |

Though DDT applications were never sufficient to suppress *Gnorimoschema* activity entirely, they promoted greater top development than occurred in untreated plots. This has again been reflected by an increase in both weight and number of tubers produced, the DDT spray (18 and 23 per cent. increases) being more effective in this respect than the dust (4 and 11 per cent.).

As expected from the habits of tuber formation of this variety, hilling, though somewhat more likely to cause plant damage under the soil conditions prevailing, has had a marked effect on both weight and number of tubers produced. Both early and late hilling caused a reduction of some 15 per cent. in weight of tubers produced.

### Trial 3.

This trial, located on a sandy loam, was planted to the variety Factor in mid-August. *Gnorimoschema* moths were present in the area by October 5, when DDT treatments were applied. The early hilling treatment was carried out on October 11, when the majority of plants were flowering. The friable soil enabled hills to be formed satisfactorily. Before the late hilling treatment was applied on November 3, the abnormal rains promoted abnormally large tops and these hills were not completed without some difficulty and damage to plants.

Moths were again prevalent and foliage damage was evident in the area by early November, but rain delayed the application of DDT until November 15. Excessive top growth and further rains reduced the efficacy of both insecticidal and cultural treatments. Slight activity and damage by *Epilachna* were observed in early November and some plant defoliation had occurred by harvest.

Late rains and excessive soil moisture delayed harvesting until November 24, when ground cracking was evident on the better drained portions of the experimental area. The results obtained are expressed as sub-plot means in Table 6.

**Table 6.**

DATA FOR 1949 EXPERIMENT (TRIAL 3).

| Insecticidal Treatments. | Cultural Treatments. 1. No Hilling. | 2. Early Hilling. | 3. Late Hilling. | Means. | Significant Differences. |
|---|---|---|---|---|---|
| | *(a) Mean weight of tubers from 10 plants (oz.)* | | | | |
| A (DDT spray) .. | 198 | 204 | 201 | 201 | |
| B (DDT dust) .. .. | 215 | 191 | 217 | 208 | |
| C (Untreated) .. .. | 243 | 196 | 184 | 208 | |
| Means .. .. .. | 219 | 197 | 201 | 205 | |
| | *(b) Mean number of tubers from 10 plants.* | | | | |
| A (DDT spray) .. | 73 | 69 | 79 | 73 | |
| B (DDT dust) .. .. | 77 | 82 | 89 | 82 | (B > A, C) |
| C (Untreated) .. .. | 80 | 68 | 73 | 74 | |
| Means .. .. .. | 77 | 73 | 80 | 77 | |
| | *(c) Mean weight of tops of 20 plants (oz.)* | | | | |
| A (DDT spray) .. | 206 | 134 | 178 | 173 | |
| B (DDT dust) .. .. | 159 | 150 | 138 | 148 | |
| C (Untreated) .. .. | 162 | 135 | 79 | 125 | |
| Means .. .. .. | 176 | 140 | 132 | 149 | |
| | *(d) Percentage tuber infestation (all grades).* | | | | |
| A (DDT spray) .. | 22·5 | 19·2 | 8·4 | 16·7 | 1 » 2, 3 |
| B (DDT dust) .. .. | 26·7 | 12·6 | 14·4 | 17·9 | C > A, B |
| C (Untreated) .. .. | 29·1 | 18·1 | 22·5 | 23·2 | |
| Means .. .. .. | 26·1 | 16·6 | 15·1 | 19·3 | |

In the last column, brackets indicate that the difference exceeds the necessary difference for significance, but the F value in the analysis of variance is not significant.

> = significantly greater than at the 5% level.

» = significantly greater than at the 1% level.

The interval of approximately six weeks between the two insecticide applications coincided with a period when crop development was proceeding rapidly. The early DDT application had little influence on pest populations during this period and all plots were subject to similar levels of pest activity. The late insecticide treatment, nine days prior to harvest, would not have

retrieved the position and thus little benefit in top weight or tuber yields could be expected from DDT applications. The late application of DDT did reduce percentage tuber infestation by approximately 30 per cent.

Tuber infestation at harvest was far in excess of what would have been expected had DDT been applied on a closer schedule. Despite the relatively high populations of *Gnorimoschema* throughout November, both early and late hilling reduced tuber infestation by approximately 40 per cent.. while a combination of late DDT spraying and late hilling was very effective in preventing tuber damage.

Hilling had no influence on tuber yield, in contrast to the finding in other experiments. The friable soil and the ease with which the hills were formed may have been partly responsible, though the habit of the variety Factor in forming the greater bulk of its tubers close to the base of the plant would preclude damage of the order associated with the long stoloned varieties, Sarenac and Sebago.

## 1950 Experiment.

Triplicate trials differing only in soil type and variety planted were commenced. One planted to the variety Katahdin on a heavy black clay loam became waterlogged soon after planting and was abandoned. Whereas all plots in the two previous season's experiments embraced four rows, plots in the 1950 series were increased in size to include five rows, the plants in the three inner rows furnishing the data recorded.

### Trial 1.

The variety Sequoia was planted on a dark-brown sandy loam on August 9, following heavy July rains. The majority of the sets had germinated by the end of August. Flowering was well advanced by October 13 and two weeks later cultural treatment (2) was applied. The late hilling treatment was carried out on November 21, when the hills in treatment (2) plots were repaired. Further cultural attention was prevented by rain.

Very slight moth activity was noticed on September 20, and a pretreatment count of larval mines on September 29 gave a mean of 0·16 mine per terminal. DDT applications were made on September 29. A further count of larval mines on October 13 gave the following results :—Sprayed plots 0·01 mine per terminal ; dusted plots 0·02 mine per terminal and untreated plots 0·10 mine per terminal. Further DDT was applied immediately following this count to check tuber moth activity in treated plots. A third estimate of *Gnorimoschema* activity on November 3 revealed the following order of infestation in plots :—Sprayed—0·0 mine per terminal ; dusted—0·01 mine per terminal ; and untreated—0·44 mine per terminal. A third and final insecticide treatment was applied following this count.

Very low populations of the two jassids *Austroasca viridigrisea* and *Orosius* (*Thamnottetix*) *argentatus* Evans were recorded in late September. Newly established colonies of the aphis *Macrosiphum gei* were prevalent at this time but these

disappeared from plants receiving DDT applications. Large numbers of *Epilachna* adults appeared in the plots in early October, but these insects largely disappeared from plots after the second DDT application, though some persisted in untreated plots until harvest.

Though rain was recorded frequently throughout October and November, the plants did not produce excessive top growth. An attack of Irish blight (*Phytophthora infestans* de Bary) developed in showery weather in early November and severe plant defoliation had occurred by the end of the month. This discouraged the development of tuber moth in the tops as the plants aged, while the persistent showers throughout November prevented ground cracking as tubers developed. As a result, tubers were harvested on December 8 virtually free from *Gnorimoschema* infestation. The yield data obtained are presented in Table 7; each value represents the mean yield for a 5-plant sample, weights being given in ounces.

**Table 7.**

Data for 1950 Experiment (Trial 1).

| Insecticidal Treatments. | Cultural Treatments. | | | | Significant Difference 5% level. |
|---|---|---|---|---|---|
| | 1. No Hilling. | 2. Early Hilling. | 3. Late Hilling. | Means. | |
| | | | *(a) Weight of tops.* | | |
| A (DDT spray) .. | 27·3 | 31·6 | 20·7 | 26·5 | |
| B (DDT dust) .. .. | 24·5 | 25·9 | 26·0 | 25·5 | A, B > C |
| C (Untreated) .. .. | 23·9 | 18·2 | 21·9 | 21·4 | |
| Means .. .. .. | 25·2 | 25·2 | 22·9 | 24·4 | |
| | | | *(b) Number of tubers produced.* | | |
| A (DDT spray) .. | 30·4 | 32·2 | 33·8 | 32·1 | |
| B (DDT dust) .. .. | 30·4 | 30·8 | 29·5 | 30·2 | |
| C (Untreated) .. .. | 31·0 | 30·8 | 31·2 | 31·0 | |
| Means .. .. .. | 30·6 | 31·3 | 31·5 | 31·1 | |
| | | | *(c) Weight of tubers.* | | |
| A (DDT spray) .. | 127·6 | 123·9 | 143·1 | 131·5 | |
| B (DDT dust) .. .. | 128·6 | 127·0 | 127·4 | 127·6 | A > C |
| C (Untreated) .. .. | 123·3 | 119·1 | 117·3 | 119·9 | |
| Means .. .. .. | 126·5 | 123·3 | 129·3 | 126·4 | |

The *Gnorimoschema* damage recorded in the tops prior to harvest was too slight to influence tuber production and any differences between treatments must be considered as due to the combined effect of all pests encountered. *Epilachna*, particularly, defoliated some plants in untreated plots and its effects would largely account for any differences between treatments in weight of tops produced. DDT treated plots were noticeably more leafy than untreated plants. Any top damage

would in turn be reflected in tuber yields, and, in particular, late damage would be more likely to influence weight of tubers produced without necessarily affecting the number of tubers formed. Hilling had no apparent effect on the number or weight of tubers produced.

**Trial 2.**

This trial, planted to the variety Sebago, was located on a dark-grey to black clay loam. Rain delayed planting until August 15.

Cultivation and irrigation were well maintained and weeds were always under control, so good even growth resulted. The early cultural treatment (2) was applied on October 25, the hills being repaired when cultural treatment (3) was applied two weeks later. All hills were fairly well maintained until harvest. The heavy soil was not entirely suited to hilling ; ground packing followed the late winter and early spring rains and the cloddy nature of the soil often prevented a uniform hill being maintained.

**Table 8.**

DATA FOR 1950 EXPERIMENT (TRIAL 2).

| Insecticidal Treatments. | Cultural Treatments. | | | | Significant Differences. |
|---|---|---|---|---|---|
| | 1. No Hilling. | 2. Early Hilling. | 3. Late Hilling. | Means. | |
| | | | *(a) Weight of tops.* | | |
| A (DDT spray) .. | 38·6 | 31·9 | 27·7 | 32·7 | A » C |
| B (DDT dust) .. .. | 32·4 | 31·7 | 28·0 | 30·7 | B > C |
| C (Untreated) .. .. | 30·6 | 25·1 | 21·3 | 25·7 | |
| Means .. .. .. | 33·9 | 29·5 | 25·7 | 29·7 | |
| | | | *(b) Number of tubers.* | | |
| A (DDT spray) .. | 30·3 | 25·2 | 27·9 | 27·8 | 1, 3 > 2 |
| B (DDT dust) .. .. | 29·9 | 25·4 | 29·9 | 28·4 | A, B > C |
| C (Untreated) .. .. | 27·4 | 21·9 | 26·9 | 25·4 | |
| Means .. .. .. | 29·2 | 24·1 | 28·3 | 27·2 | |
| | | | *(c) Weight of tubers.* | | |
| A (DDT spray) .. | 126·9 | 114·8 | 109·9 | 117·2 | |
| B (DDT dust) .. .. | 112·5 | 106·9 | 122·8 | 114·1 | A > C |
| C (Untreated) .. .. | 118·2 | 99·0 | 103·5 | 106·9 | |
| Means .. .. .. | 119·2 | 106·9 | 112·1 | 112·7 | |
| | | | *(d) Percentage tuber infestation (all grades).* | | |
| A (DDT spray) .. | 3·3 | 0·5 | 0·3 | 1·1 | 1 » 2, 3 |
| B (DDT dust) .. .. | 15·1 | 0·8 | 3·0 | 4·8 | B, C » A<br>with 1 : B, C » A |
| C (Untreated) .. .. | 11·6 | 1·9 | 1·3 | 4·0 | with 2 : no sig. diff.<br>with 3 : B » A |
| Means .. .. .. | 9·3 | 1·0 | 1·3 | 3·0 | |

**Table 8.**—*continued.*

DATA FOR 1950 EXPERIMENT (TRIAL 2).

| Insecticidal Treatments. | Cultural Treatments. 1 No Hilling | 2 Early Hilling. | 3 Late Hilling. | Means. | Significant Differences. |
|---|---|---|---|---|---|
| *(e) Percentage tuber infestation (first grade).* | | | | | |
| A (DDT spray) .. .. | 2·6 | 0·1 | 0·8 | 0·9 | 1 » 3 ; 1 > 2 |
| | | | | | with 1 : B » A |
| B (DDT dust) .. .. | 9·8 | 1·0 | 0·1 | 2·2 | with 2 : C > A |
| C (Untreated) .. .. | 5·6 | 2·4 | 0·3 | 2·2 | with 3 : no sig. diff. |
| Means .. .. .. | 5·7 | 0·9 | 0·3 | 1·7 | |
| *(f) Percentage tuber infestation (second grade).* | | | | | |
| A (DDT spray) .. | 2·5 | 1·4 | 0·0 | 0·9 | 1 » 2, 3 |
| | | | | | B » A ; C > A |
| | | | | | with 1 : B, C » A |
| B (DDT dust) .. .. | 13·5 | 0·8 | 5·9 | 5·5 | with 2 : no sig. diff. |
| C (Untreated) .. .. | 18·6 | 0·4 | 0·5 | 3·7 | with 3 : B » A ; B > C |
| Means .. .. .. | 10·3 | 0·8 | 1·1 | 3·0 | |
| *(g) Percentage tuber infestation (chats).* | | | | | |
| A (DDT spray) .. | 1·2 | 0·0 | 0·0 | 0·1 | 1 » 2, 3 |
| | | | | | B, C » A |
| B (DDT dust) .. .. | 21·8 | 0·1 | 3·6 | 5·5 | with 1 : B, C » A |
| | | | | | with 2 : no sig. diff. |
| C (Untreated) .. .. | 11·9 | 0·9 | 2·2 | 3·9 | with 3 : B > A |
| Means .. .. .. | 9·7 | 0·2 | 1·3 | 2·5 | |

> = significantly greater than at 5% level.

» = significantly greater than at 1% level.

*Gnorimoschema* moths were active soon after the plants appeared through the ground in late August. Little larval damage had occurred by the end of September, when a count of larval mines in 360 terminals showed a mean of 0·3 per terminal. DDT was applied to treatment plots on September 29, October 13, and November 2, immediately following counts of larval mines in the terminals. The results of these counts are presented in Fig. 3. By early November, extensive leaf damage had occurred in untreated plots, individual plants showing up to 11 larval mines per 6-leaved terminal.

Leaf damage due to *Austroascus viridigrisea* was evident soon after the crop appeared through the ground and increased in untreated plots until late in November. Colonies of *Macrosiphum gei* were present in early September, but disappeared later. Adults of *Epilachna 28-punctata* caused leaf damage in untreated plots by mid-October and had reinfested the DDT treated plots by harvest, but damage was of little consequence. Leaf defoliation had occurred on some plants in untreated plots by this time.

Though this soil type is prone to crack as it dries out, no noticeable cracks had occurred by early November. Slight cracking was noticeable when plots were harvested on December 12. The yield data from this trial are set out in Table 8, values being expressed as the mean for a 5-plant sample and the weights given in ounces.

Three DDT sprayings prevented larval infestation in the tops until early November and maintained a residual action sufficient to prevent serious reinfestation before harvesting. Though the DDT dust checked larval mining until early November, an appreciable larval population had developed in these plots by harvest. Larval activity was evident in untreated plots by early November (Fig. 3). Leaf mining increased rapidly later in the month and many plants had been largely defoliated when the plots were harvested. Differences between the number of mines counted per terminal for treatments at the final sampling on December 1 were significant at the 1% level.

Coupled with the effects of other pests in untreated plots, the leaf injury caused by *Gnorimoschema* reduced top growth considerably. This obvious effect was confirmed at harvest, when values for mean weight of tops produced showed increases of 27 and 19 per cent. for DDT spray and dust respectively. By protecting top growth, DDT, particularly in spray form, has greatly increased both number and weight of tubers produced. Spray and dust applications were equally effective in ensuring tuber formation, but the poorer residual action of the dust (Fig. 3), by allowing some top reinfestation while tubers were developing, resulted in a lower weight of tubers at harvest than was obtained in sprayed plots.

There is a suggestion (Table 8) that late hilling in particular has reduced top growth, but differences are not significant. It is to be expected that the lower leaves and prostrate stems would be covered by soil thrown up by implements, and this in itself would account for any reduction in top weight in late hilled plots. The cutting of surface roots may check top development also, but probably would be of lesser importance in the final results. Significant differences were obtained between the number of tubers produced in early hilled plots and those from plots receiving the other cultural treatments. These findings are also reflected in the weight of tubers produced, but differences are not significant.

Three applications of DDT spray, by preventing larval infestation in the tops, considerably reduced the likelihood of tuber infestation. In unhilled plots, only 3·3 per cent. of the tubers were infested, while hilling reduced this figure to less than 1 per cent. This benefit from spray application was reflected in the results for all three grades of tubers. DDT dust alone was of little benefit in preventing tuber infestation, as moths were able to reinfest plots before harvest.

Hilling alone reduced tuber infestation from 11·6 per cent. to less than 2 per cent. of the total tubers produced, with early and late hilling giving equal benefit in this respect. Such protection could not have been expected had harvesting been delayed.

Irrespective of treatment, the infestation recorded in first grade tubers was always lower than that for the other two grades.

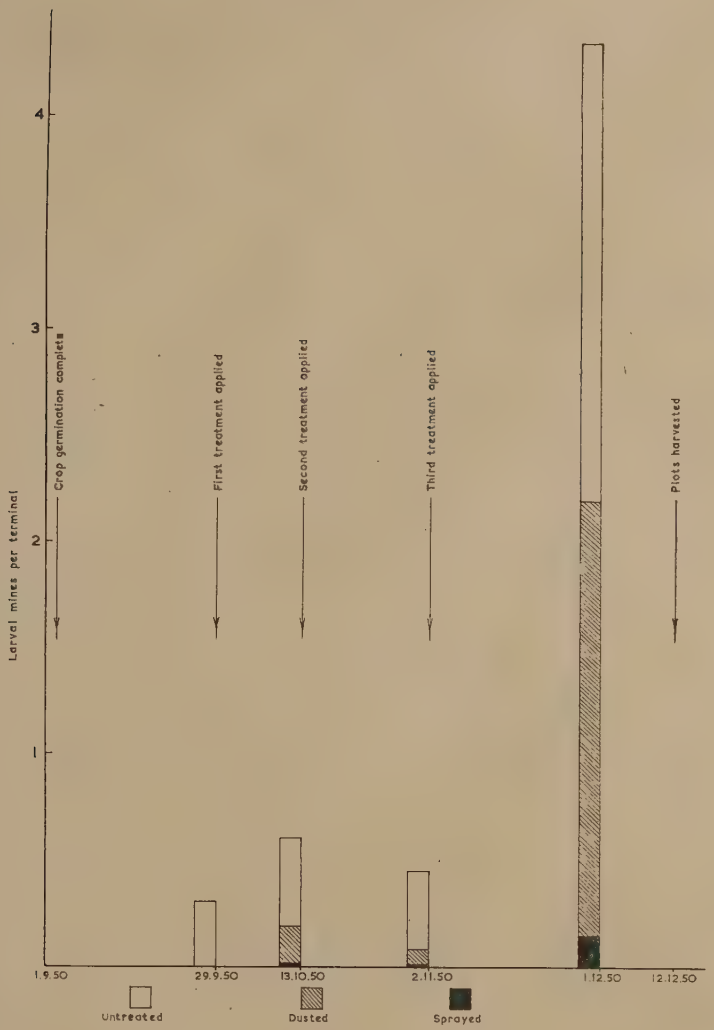


Fig. 3.

Diagram of Larval Mines per Terminal on Various Sampling Dates (Trial 2, 1950).

## GENERAL DISCUSSION.

In the several experiments concluded, the value of both DDT and hilling in checking the ravages of *Gnorimoschema* was established repeatedly. Each was shown to fulfil a definite role in the control programme and the information obtained has suggested how each can best be applied in the field to benefit both *Gnorimoschema* control and general crop production.

### The Role of DDT Applications.

*Formulation of DDT Used.*—At the concentration used, DDT spray was more effective than DDT dust in protecting the tops. Helson (1949) also came to this conclusion, though he found that dust applications were effective in late season crops, where populations waned as the season progressed.

Spray applications reduced moth populations rapidly and destroyed most larvae in their mines. Dusts were not as effective in these respects. Spraying was far superior in preventing larval mining in the tops in both 1950 trials, while reinfestation occurred much more rapidly in dusted plots once treatments were discontinued (Fig. 3). Variability in top damage between dusted plots suggested a less even cover of insecticide.

Figures for tuber infestation at harvest reflect the value of both methods of DDT application. In trial 2 of 1950, the infestation in dusted plots (15·1 per cent.) was significantly higher than in sprayed plots (3·3 per cent.). In fact, values for dusted plots were comparable with those for untreated plots, providing further evidence of the poor residual action of DDT dusts.

On a cost basis, spray is to be preferred to dust. Even if dusting was as effective as spraying, using 1 lb. active constituent per acre, the cost of dust would prove almost twice as much as that of spray.

*Effect on Crop Development.*—In the experiments reported, the weight of tops produced on sprayed plots was significantly greater than that on untreated plots. Greater differences occurred when early applications of DDT were necessary. Though the prevention of damage by *Gnorimoschema* larvae was largely responsible for these differences, the control of other potato pests by DDT would also contribute towards increased top development. The jassids *Austroasca viridigrisea*, *A. alfalfae* Evans and *Orosius* (*Thamnotettix*) *argentatus*, together with the potato aphis (*Macrosiphum gei*) were recorded from practically all experiments soon after germination and could be expected to influence the rate of early crop development, while the leaf eating ladybird (*Epilachna 28-punctata*), though rarely numerous before flowering, caused severe leaf damage in untreated plots before harvest.

Granovsky (1944) showed that DDT would prove effective against most insect pests of potatoes and these findings were borne out in these experiments. All the pests mentioned above were controlled following DDT applications and top growth could develop without check from insect attack.

Plots receiving adequate DDT spray applications made rapid and sustained growth and a comparison with untreated plants indicated that DDT treatment had stimulated plant development. Protection from the general insect population was reflected in the increase in both number and weight of tubers produced. Bald and Helson (1944) found that the yield of infected plants was approximately proportional to the amount of leaf area left undamaged by *Gnorimoschema* larvae. Hofmaster (1949) and Lloyd (1951) also suggested that control of *Gnorimoschema* in the tops should result in increased yields. Maximum increases in yield, apart from a greater percentage of sound tubers following pest control in the tops, can be expected to occur when the initial DDT application is made in the early stages of crop development.

*Timing and Rate of Application.*—Caldwell (1946) recommended that DDT treatment should commence with flowering, while Lloyd (1951) recommended application at the first sign of pest activity.

In the 1948 and 1950 experiments, DDT applied at the first indication of pest activity and followed by further applications as required was effective in preventing foliage injury for the greater period of crop growth, thus allowing maximum tuber development. Treatment applied late in the life of the crop may destroy populations before harvest but does not prevent foliage damage and its effect on yield. This was shown to be the case with the preharvest treatment in the 1948 experiment, and to a slight degree with the late treatment in trial 3 of the 1949 experiment.

Two applications of spray—the first when plants had only four to six leaves and the second 10 days later—were sufficient to control the pest during the 1948 experiment, the check to insect populations and associated stimulation of top growth being still evident at harvest. In the 1949 experiment, though 1 lb. of DDT per acre was applied at each application, the interval between applications was prolonged by rain to from four to six weeks and allowed *Gnorimoschema* to cause top damage in the interval between sprays and persist in sufficient numbers to damage tubers at harvest. For the 1950 experiments, three sprays with intervals of two to three weeks between applications gave excellent control in the tops and virtually prevented tuber infestation. It is possible that the third application was not necessary for additional top protection, though its benefit was reflected in the greater percentage of sound tubers harvested.

The results for the 1948 experiment were obtained when *Gnorimoschema* populations were high throughout the Lockyer Valley. Hofmaster (1949) found that two applications, 10 days apart, each applied at the rate of 1 lb. DDT per acre, were sufficient to control heavy infestations. Leaf mine counts in the 1950 experiment suggested that under the conditions of the experiment, DDT applied at the rate of 1 lb. per acre maintained its effectiveness for at least two weeks after application. Cannon (1948) argued that, at the same rate of application, the interval between treatments may extend up to three weeks.

Caldwell (1946) recommended three or four applications at fortnightly intervals, commencing with flowering. For effective top protection, earlier treatment is generally necessary and it seems that, if DDT is first applied when populations are low (preferably soon after crop germination), only two (or at the most, three) applications of DDT are necessary to cope with *Gnorimoschema* in seasons that favour its development. This schedule should be adopted as a routine measure, the application of the third treatment being dictated by the likelihood of further damage before harvest.

## The Role of Hilling.

No detailed evaluation of hilling methods was undertaken. Lloyd (1943) investigated this point and found that it is necessary to ensure that the soil is well thrown up along the rows of plants so that all tubers are adequately covered with at least two inches of soil (Fig. 4). Though hilling is generally adopted by most potato growers in southern Queensland, the time of its application varies. Many growers hill their rows at or soon after flowering (Cartmill and Bechtel, 1951), though Lloyd (1946) recommended late hilling, up to 14 weeks after planting, when the tubers are commencing to swell and crack the ground.

*Prevention of Tuber Infestation.*—Hilling was shown to play an important part in protecting tubers from *Gnorimoschema* damage (Fig. 4). Langford (1933) was able to reduce tuber infestation from 18·27 per cent. to 5·5 per cent. by hilling alone, while Lloyd (1950) gave the figures 14·9 per cent. for unhilled and 4·2 per cent. for hilled plants. In trial 2 of 1950, tuber moth infestation was reduced

Fig. 4.

Potatoes Effectively Hilled Against Tuber Infestation by *Gnorimoschema operculella* (Zell.).

from 11·6 per cent. to 1·3 per cent. by late hilling, this difference being significant at the 1 per cent. level. Despite insecticidal applications, populations are not always entirely destroyed and some tuber infestation will occur in unhilled crops. Hilling, apart from controlling weed growth, seals cracks in the soil and, if properly applied, effectively checks *Gnorimoschema* damage until the crop can be harvested.

Hilling proves exceedingly beneficial if harvesting is unavoidably delayed. This effect was shown in trial 2 of the 1949 experiment, where a significantly lower infestation was still evident in late hilled plots despite major tuber moth activity over the experimental area following a delay of almost three weeks in harvesting. Hilling was responsible for even greater differences in tuber infestation in trial 3 of the same experiment, though only a slight delay in harvest had occurred.

Despite well formed hills, varieties such as Sarenac and Sebago that produce their crop on elongated stolons often have a percentage of their tubers either wholly exposed or only lightly covered with soil after the hills are formed. Hilling cannot be expected to eliminate tuber infestation entirely in these varieties, and appreciable damage may occur if harvesting is delayed.

Evidence was obtained (trial 2 of 1949 and trial 2 of 1950) that first grade tubers are less subject to infestation than tubers of smaller size. The majority of first grade tubers are formed early and thus are found deeper in the soil. Chats are formed later in the sequence of crop formation and lie close to the soil surface ; they are protected from *Gnorimoschema* damage only if a perfect hill is formed. First grade tubers provide the bulk of the total crop produced, and the benefit derived from hilling is further appreciated if other grades are left out of consideration.

*Influence on Yield.*—The results from these experiments suggest that a definite time interval between planting and hilling is desirable if the operation is not to interfere with yield. The early hilling treatment was applied soon after flowering, at a time when many tubers were being formed, while late hilling was designed to avoid this important period of crop formation. Often these treatments could not be applied at the intended time, due either to rain or to other unavoidable causes. A summary of the relevant data from the various experiments (Table 9) shows the relationship between time of hilling and tuber yield.

Disregarding varietal effects, hilling reduced yield when applied soon after flowering, or within 10 weeks of planting. Beyond this time interval, damage decreased until no depressing effect was apparent after an interval of 15 weeks. It would appear that early hilling, under certain circumstances, interferes with the formation of the tubers, but it is also likely to retard tuber development by temporarily checking plant growth.

Lloyd (1950) recorded a loss of up to 10 per cent. by weight due to hilling alone, and this finding was supported by these experiments (cf. trial 2 of 1949). The extent of the damage incurred depended not only on the age of the crop when hilling was carried out but also on the variety of potato being hilled. Hilling depressed the yield of Factor when applied up to 11 weeks after planting, but this

**Table 9.**

DATA SHOWING RELATIONSHIP BETWEEN TIME OF HILLING AND YIELD.

| Variety. | Early Treatment. | | | Late Treatment. | | |
|---|---|---|---|---|---|---|
| | Weeks after planting. | Percentage decrease in tuber yield. | | Weeks after planting. | Percentage decrease in tuber yield. | |
| | | Number. | Weight. | | Number. | Weight. |
| | | | 1949 *Experiment.* | | | |
| 1. Sebago | .. | .. | .. | 15 | .. | .. |
| 2. Sarenac | 8 | 13† | 17* | 11 | 24* | 14† |
| 3. Factor | 8 | .. | 11‡ | 11 | .. | 9‡ |
| | | | 1950 *Experiment.* | | | |
| 1. Sequoia | 11 | .. | .. | 15 | .. | .. |
| 2. Sebago | 10 | 21† | 11‡ | 12 | 3† | 7‡ |

* = sig. at 1 % level; † = sig. at 5 % level; ‡ = not significant.

decrease was not significant and was recorded for weight of tubers only. Sequoia showed no apparent reduction in yield when early and late hilling were applied 11 and 15 weeks respectively after planting. Considerable reduction in both number and weight of tubers was recorded for the variety Sarenac, though hilling times corresponded with those for Factor. Despite its application at greater time intervals after planting, hilling caused more damage to Sebago than to Factor.

Lloyd (1946) suggested that drying out of the soil following hilling was the main cause of yield reduction. This would not be an important consideration here, as all experiments were grown under irrigation and at no time during crop development was soil moisture inadequate. Hilling implements, by cutting feeding roots and mechanically damaging tops, could be expected to interfere with normal tuber development. Such damage would be reflected largely in a decreased weight of tubers at harvest.

Reduction in the number of tubers formed following hilling can be directly associated with the habits of the several varieties grown. Sequoia and Factor produced their tubers close to the base of plants. Both Sarenac and Sebago form their tubers on long stolons, and tubers were located in the soil up to 10 inches from the base of plants of the former variety. Hilling implements could be expected to destroy or damage many tubers of these two varieties.

*Time of Application.*—The spring potato crop in southern Queensland is usually ready for harvest in 16 to 17 weeks from the date of planting. From the evidence obtained (Table 9) it would seem that, to avoid undue crop damage, hilling should be carried out no earlier than 12 weeks from planting, though an extension of this time to 14 weeks, as recommended by Lloyd (1946), would obviate any possibility of damage to most varieties. If tubers are to be adequately protected from *Gnorimoschema* attack, hilling cannot be delayed later than 14 weeks from planting. The increased yield of sound tubers due to hilling alone will more than compensate for any depression in total yield that may occur when hilling is applied later than 12 weeks from planting.

## CONCLUSIONS.

A schedule of DDT applications is essential for effective control of *Gnorimoschema* in the spring potato crops in southern Queensland. Two (or in extreme cases, three) applications of spray, each at the rate of 1 lb. DDT per acre, will prevent pest populations developing for the greater period of crop growth and so prevent any check to normal tuber formation and development. Applications should commence at the first sign of pest activity within the crop and be repeated at fortnightly intervals. If treatments are properly applied, their cost will be more than compensated by the extra yield of tubers obtained and the increased percentage of sound tubers at harvest.

The above schedule of DDT applications will not eliminate or prevent re-entry of *Gnorimoschema* populations late in the period of crop development. Hilling the plants between 12 and 14 weeks after planting will prevent effectively any likelihood of tuber infestation in the preharvest period. The hills should be constructed so that all tubers are well covered with soil and should be maintained against weathering during late rains or irrigation.

Hilling alone, without early DDT spraying, cannot be expected to protect tubers from *Gnorimoschema* damage, but can be regarded only as a supplementary measure for additional tuber protection and as a safeguard against crop reinfestation or delays in harvesting. Unlike DDT spraying, hilling does not ensure maximum yields.

Hilling does not entail any additional cost to the grower, as it is a normal and long established practice of potato culture in southern Queensland. However, every effort should be made to achieve correct timing.

As stated earlier, observation plots were established during the spring of 1951 to demonstrate the effectiveness of control measures recommended against *Gnorimoschema*. The recommendations followed were those published in an extension article (May, 1951) and based chiefly on work conducted during the previous three years and now reported in this paper. Extremely dry weather prevailed throughout spring and early summer and tuber moth was of more than usual importance in the area. Crops receiving inadequate DDT applications suffered heavily from tuber moth attack and poor yields of sound tubers were recorded. Where insecticidal treatment was applied early and continued in accordance with recommendations, tops developed normally. Late hilling adequately prevented damage prior to harvest, for less than 5 per cent. of harvested tubers were infested. These findings support the conclusions drawn from the trial data presented in this paper.

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